

# THE LANCET

## Global Health

### **Supplementary appendix**

This appendix formed part of the original submission and has been peer reviewed.  
We post it as supplied by the authors.

Supplement to: Humphrey JH, Mbuya MNN, Ntozini R, et al. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on child stunting and anaemia in rural Zimbabwe: a cluster-randomised trial. *Lancet Glob Health* 2019; **7**: e132–47.

## **SUPPLEMENTARY APPENDIX**

### **Independent and combined effects of improved water, sanitation and hygiene, and improved complementary feeding, on child stunting and anaemia in rural Zimbabwe: a cluster-randomised trial**

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## **APPENDIX 1**

### **SUPPLEMENTARY METHODS**

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## **SUPPLEMENTARY METHODS**

### **a) Randomisation procedure**

A highly constrained randomisation technique was used to allocate clusters (stratified by district) to treatments. We randomly selected 1000 allocations from among 5000 computer-generated allocations that balanced the 4 treatment arms on 14 parameters without pre-specified bounds. From the 1000, we randomly selected 10 for a public randomisation ceremony; Figure S1. Each randomisation scheme divided the randomisation units into 4 groups of approximately 53 units. Each scheme's corresponding colour-coded map was printed on a separate sheet and displayed at a public forum attended by all elected councillors from the study area, District and Provincial Administrators, and Ministry of Health and Child Care authorities. In their presence, 10 plastic balls (numbered 1-10) were placed in an opaque sack. A community representative selected one ball from the sack, thereby identifying which of the 10 numbered allocations would be used. Then, four balls (labelled A, B, C, and D) were placed in one sack, and four balls (labelled with the 4 treatment arms) were placed in a second sack. Representatives drew a ball from the first sack and a ball from the second sack, pairing a group of clusters with one of the four treatment arms, thereby mimicking a widely known World Cup draw procedure. This was repeated twice more to pair the next two groups of clusters with two more treatment arms. The remaining balls formed the final pairing. This second stage was included to provide an additional assurance of impartiality/randomness and a further opportunity for participation of the community leadership.

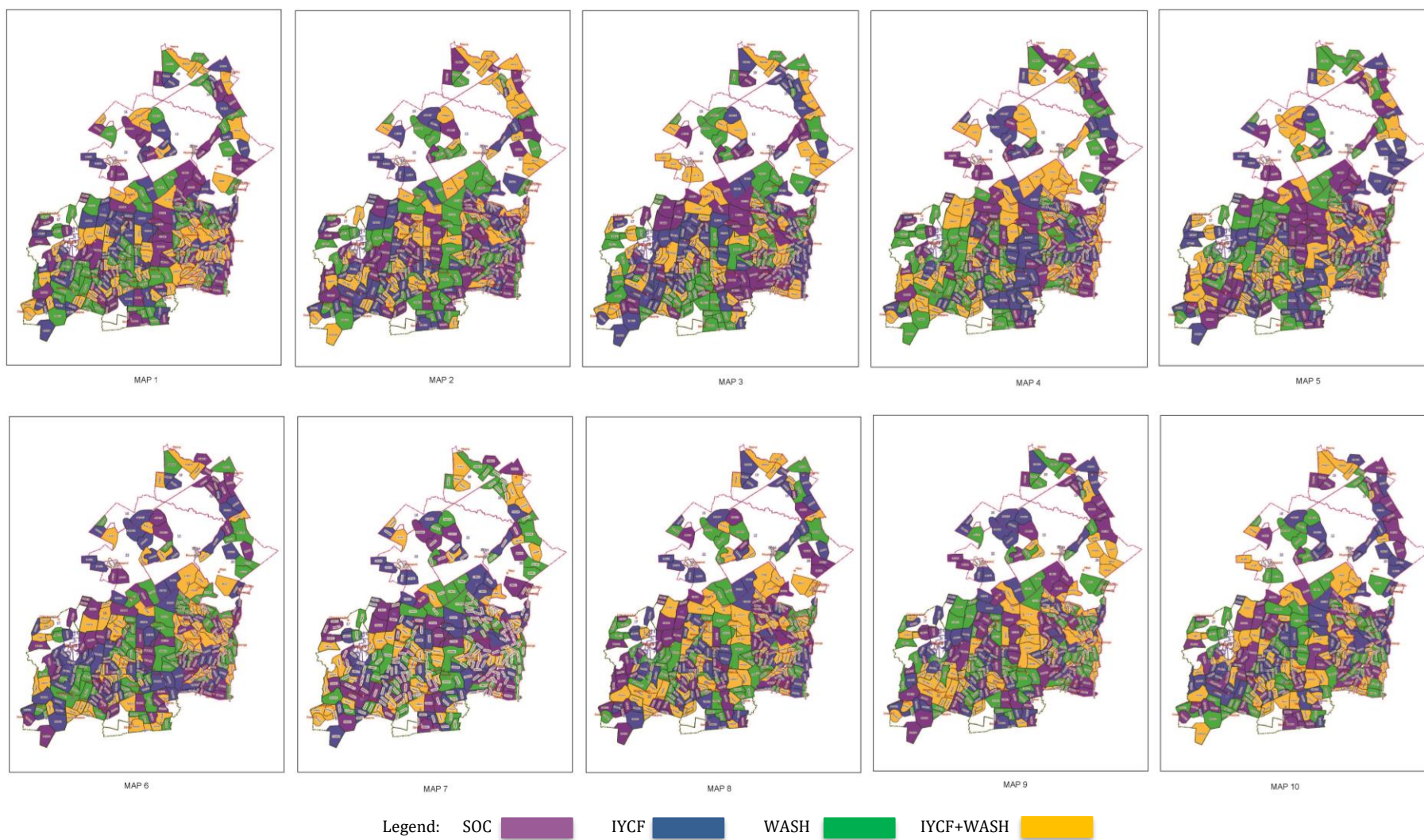


Figure S1. Maps of ten SHINE randomization schemes

## **b) Changes in gestational age enrolment criteria**

The goal at the start of the trial was to recruit women between 10-14 weeks gestation, so women more than 14 weeks gestation were excluded. However, this cut-off was liberalised three times over the life of the trial to maximise recruitment because many women were excluded due to pregnancy that was >14 gestational weeks at screening. The upper limit of gestational age was therefore increased to 18 weeks (August 22, 2013), 24 weeks (January 3, 2014), and any time prior to parturition (October 20, 2014), through trial protocol amendments.

## **c) Further details of interventions**

*Standard of Care (SOC) Intervention:* Village Health Workers were trained through the Ministry of Health and Child Care curriculum, which instructs VHWs to visit pregnant women and infants frequently, although the precise content or purpose of each visit is not specified. Consequently the SHINE SOC intervention was designed to standardize the number of visits (3 antenatal and 12 postnatal visits) and the content of primary health care messages across treatment arms. Four of these visits promoted exclusive breastfeeding (EBF) from birth to 6 months using modules designed to overcome contextual barriers identified in formative work. Other SOC modules include prevention of mother-to-child HIV transmission (PMTCT), antenatal care, hospital-based delivery, family planning and immunizations.

*WASH Intervention:* Within 6 weeks of enrolment (~20 weeks gestation) into the WASH and IYCF+WASH arms of the trial, a Blair Ventilated Improved Pit (VIP) Latrine was constructed at the participant's household and two 'Tippy Tap' hand-washing stations (locally manufactured, and adapting the model piloted by the Kenya WASH Benefits trial) were installed near the latrine and kitchen; Figure S2.



Figure S2: Blair VIP latrine and Tippy Tap

WASH Modules 1 (delivered at 24 gestational weeks) and 2 (32 gestational weeks) promoted safe disposal of faeces, and hand-washing with soap after faecal contact and before food preparation and eating, respectively. Our intention was for the baby to be born into a household in which latrine use and household hand-washing behaviours were normalised and habitual. WASH Module 3 (protecting babies from faecal ingestion during exploratory play) was delivered when the baby was 2 months old; a washable 2.8m x 3.0m locally manufactured mat and plastic play yard (North States, Minneapolis MN) were provided at 2 months and 6 months, respectively; Figure S3.





Figure S3: SHINE mat and play yard

WASH Module 4 (treat all drinking water given to babies after 6 months of EBF) was delivered at 4 months of age, along with point-of-use chlorination (WaterGuard: a dilute sodium hypochlorite solution, manufactured locally by Nelspot). Liquid soap and Water Guard were regularly replenished from time of introduction (Module 2 and 5, respectively) until the infant was 18 months old. WASH Module 5, delivered at 5 months of age, stressed the importance of freshly preparing or fully reheating all foods fed to infants. A review module was delivered at 12 months.

*IYCF Intervention:* IYCF Module 1 (delivered at 5 months) linked good infant feeding to child growth, health, and intelligence. IYCF Module 2 (6 months) promoted feeding nutrient-dense food, including 20 g per day of the lipid-based nutrient supplement (LiNS) developed by the International Lipid-Based Nutrients Supplements Project, provided monthly when the baby was 6 to 18 months of age. Module 3 (7 months) was a participatory cooking demonstration in which any available household food was prepared and fed to the baby,



stressing three messages from formative research: 1) an infant can eat any food that an adult eats; 2) food should be ground so that the infant can swallow and digest it; 3) food that is locally available is important for the infant. Module 4 (8 months) promoted responsive feeding during illness, Module 5 (9 months) promoted diet diversity, and a review module was delivered at 12 months.

#### **d) SHINE trial outcomes**

##### *Primary outcomes*

- Length-for-age Z score at 18 months
- Hemoglobin at 18 months

##### *Secondary outcomes*

- Stunting (LAZ <-2) at 18 months
- Severe stunting (LAZ <-3) at 18 months
- Anemia (hemoglobin <105 g/L) at 18 months
- Severe anemia (hemoglobin <70 g/L) at 18 months
- Weight-for-age Z score (WAZ) at 18 months
- Underweight (WAZ <-2) at 18 months
- Weight-for-length Z score (WLZ) at 18 months
- Wasting (WLZ <-2) at 18 months
- Head circumference-for-age Z score at 18 months
- Mid-upper arm circumference-for-age Z score at 18 months
- 7 day maternal recall of child diarrhea, dysentery and acute respiratory infection at 12 and 18 months
- Mortality through 18 months

*Tertiary outcomes* (to be presented in subsequent manuscripts)

- Anthropometry (LAZ, WAZ, WLZ, MUAC, head circumference) at 1, 3, 6 and 12 months
- Hemoglobin at 3, 6 and 12 months (EED substudy only)
- Incidence and prevalence of diarrhea
- Persistent diarrhea
- Incidence and prevalence of acute respiratory infection
- Incidence and prevalence of fever
- All-cause and cause-specific hospitalization
- All-cause and cause-specific clinic attendance
- Adverse events and serious adverse events related to trial interventions

#### **e) Details of anthropometry measurements**

Anthropometry was undertaken by trained nurses according to Standard Operating Procedures following one week of specific residential training, which formed part of their 14-week training course undertaken prior to the start of the trial. Recumbent infant length was measured to the nearest 0.1cm using a Seca 417 infantometer (Weigh & Measure LLC., Olney, MD, USA); at the 18-month endpoint visit, length was measured three times and the median value used in analysis. Infant weight was measured to the nearest 10g using a Tanita BD-590 infant scale (Weigh & Measure LLC., Olney, MD, USA). Mid-upper arm circumference was measured on the left side to the nearest 0.1cm using a multi-purpose ShorrTape (Weigh & Measure LLC., Olney, MD, USA). Head circumference was measured to the nearest 0.1cm using a multi-purpose ShorrTape (Weigh & Measure LLC., Olney, MD, USA). Nurses underwent 6-monthly standardisation exercises, in which the same ten mothers and ten children were measured by each nurse, and values compared to a gold standard anthropometrist. A coefficient of reliability (R) value against the gold standard anthropometrist was calculated and nurses with R values <0.95 for intra- and inter-observer accuracy were given further training and additional supervision in the field. Once the nurse supervisor was satisfied that anthropometry technique had improved, the nurse resumed independent measurements. Supervisors observed every nurse conducting a research visit at least quarterly and conducted spot checks with study participants to evaluate performance.

#### **f) Definition of per protocol analysis**

Secondary modified per protocol analyses were conducted with these restrictions:

##### **WASH intervention**

Higher Fidelity: received all 5 WASH modules and 12-month review

Lower Fidelity: received less than all 5 WASH modules and 12-month review

##### **IYCF intervention**

Higher Fidelity: received all 5 IYCF modules and 12-month review

Lower Fidelity: received less than all 5 IYCF modules and 12-month review

#### **g) Distribution of length-for-age Z score at 18 months of age among infants according to group**

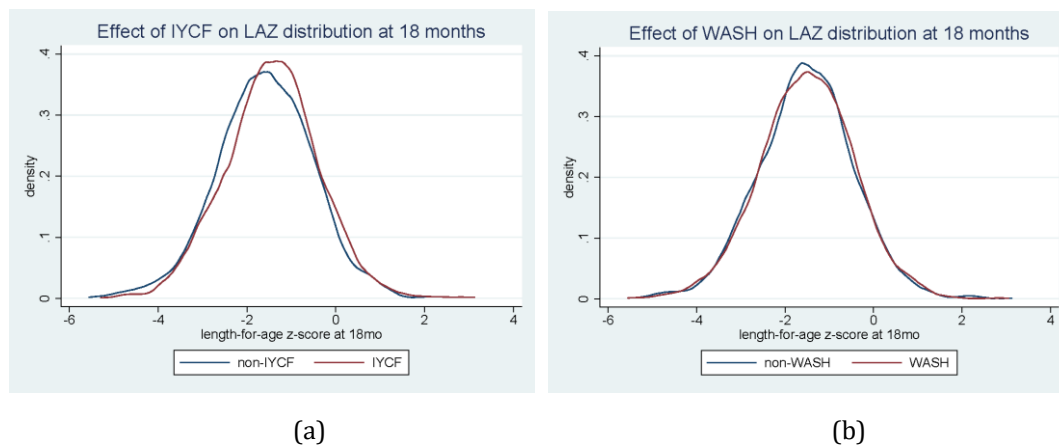


Fig S4: Distribution of length-for-age Z score at 18 months of age among infants in the (a) IYCF groups compared to the non-IYCF groups and among infants in the (b) WASH groups compared to the non-WASH groups

**Supplementary Table 1. Maternal, household, and infant baseline characteristics of HIV-negative mothers and their live born infants who completed and defaulted the 12 month visit.**

	Completed 12-month Visit	Defaulted 12-month Visit	p value
Mothers	2778	976	
Infants	2809	984	
Mothers completing baseline visit <sup>1</sup>	2627	880	
<b>Household characteristics</b>			
Size median(IQR)	5 (3 - 6)	5 (3 - 7)	0.504
<i>Wealth Quintile<sup>2</sup>:</i>			
Lowest	16.93 (16.9%X)	22.61(22.6%X)	
Second	514/2611 (19.7%)	155/867 (17.9%)	
Middle	553/2611 (21.2%)	156/867 (18.0%)	0.001
Fourth	559/2611 (21.4%)	169/867 (19.5%)	
Highest	543/2611 (20.8%)	191/867 (22.0%)	
<i>Electricity</i>			
Power grid	67/2610 (2.6%)	32/865 (3.7%)	<0.0001
<i>Other power sources</i>			
Solar	1820/2610 (69.7%)	555/865 (64.2%)	
Generator	81/2610 (3.1%)	32/865 (3.7%)	0.027
No electricity	709/2610 (27.2%)	278/865 (32.1%)	
<i>Sanitation</i>			
Household members who openly defecate (all)	5884/12094 (48.7%)	1796/3769 (47.7%)	0.812
Any latrine at household	934/2570 (36.3%)	336/855 (39.3%)	0.125
Improved latrine at household	816/2567 (31.8%)	294/854 (34.4%)	0.181
Improved latrine with well-trodden path	727/2567 (28.3%)	252/854 (29.5%)	0.535
Improved latrine with well-trodden path and not shared	634/2478 (25.6%)	223/830 (26.9%)	0.484
<i>Water</i>			
Main source of HH drinking water is improved	1637/2582 (63.4%)	545/863 (63.2%)	0.903

Treat drinking water to make it safer	323/2539 (12.7%)	107/852 (12.6%)	0.904
One-way walk time to fetch water (min), median (IQR)	10 (5 - 20)	10 (5 - 25)	0.002
Per capita water volume collected in past 24 h (L), $\bar{x}$ (SD)	9.7 (10.6)	9.7 (9.20)	<0.0001
<i>Hygiene</i>			
Hand-washing station at household	213/2451 (8.7%)	74/815 (9.1%)	0.757
Hand-washing station with water	75/2446 (3.1%)	27/814 (3.3%)	0.696
Hand-washing station with water and rubbing agent at HH	20/2446 (0.8%)	5/814 (0.6%)	0.559
Improved floor <sup>3</sup>	1437/2582 (55.7%)	469/849 (55.2%)	0.846
Number of chickens, median (IQR)	6 (2 - 10)	6 (1 - 10)	0.002
Livestock in home	1008/2609 (38.6%)	301/864 (34.8%)	<0.0001
Faeces observed in yard	827/2596 (31.9%)	259/859 (30.2%)	0.333
<i>Diet quality and food security</i>			
Household meets minimum Diet Diversity Score <sup>4</sup>	963/2310 (41.7%)	255/752 (33.9%)	<0.0001
Coping strategies Index <sup>5</sup> , median (IQR)	1 (0 - 7)	1 (0 - 6)	0.748
<i>Maternal characteristics</i>			
Age (y), $\bar{x}$ (SD)	26.4 (8.69)	23.6% (5.59)	<0.0001
Height (cm), $\bar{x}$ (SD)	160 (8.6)	159.3 (7.7)	<0.0001
MUAC (cm), $\bar{x}$ (SD)	26.6 (3.5)	26.06 (3.08)	<0.0001
<i>Schistosoma haema</i> , microscopy positive	268/2515 (10.7%)	97/790 (12.3%)	0.368
Completed schooling (y), $\bar{x}$ (SD)	9.6 (2.1)	9.7 (2.1)	0.024
Parity, median (IQR)	2 (1 - 3)	1 (0 - 2)	<0.0001
Married	2544/2638 (96.4%)	839/908 (92.4%)	<0.0001
Employed	226/2607 (8.7%)	70/867 (8.1%)	<0.0001
<i>Religion:</i>			
Apostolic	1259/2657 (47.4%)	414/914 (45.3%)	0.309
Other Christian	1196/2657 (45.0%)	418/914 (45.7%)	0.309
Other	202/2657 (7.6%)	82/914 (9.0%)	
<i>Infant characteristics</i>			
Female			0.552

Birth weight (kg), $\bar{x}$ (SD)	1393/2809 (49.6%)	496/975 (50.9%)	<0.0001
Birth weight <2500 g	3.11 (0.52)	3.08 (0.45)	0.536
Institutional delivery	205/2626 (7.8%)	70/821 (8.5%)	0.305
Vaginal delivery	2347/2620 (89.6%)	727/823 (88.3%)	0.488

<sup>1</sup> Maternal and household baseline data collected about 2 weeks after consent (~14 weeks gestation). Baseline for infants was at birth. Values are %, unless noted. For variables where [n] is not stated, <3% of data are missing based on number of baseline visits completed.

<sup>2</sup> Chasekwa B, Maluccio JA, Ntozini R, Moulton LH, Wu F, Smith LE, et al. Measuring wealth in rural communities: Lessons from the Sanitation, Hygiene, Infant Nutrition Efficacy (SHINE) trial. PLoS ONE. 2018; 13(6): e0199393.

<sup>3</sup> Improved floor defined as concrete, brick, cement, or tile. Unimproved floor defined as mud, earth, sand, or dung.

<sup>4</sup> FAO, FHI 360. Minimum Dietary Diversity for Women: A Guide for Measurement. Rome: FAO. 2016.

<sup>5</sup> Maxwell D, Watkins B, Wheeler R, Collins G. The Coping Strategy Index: A tool for rapid measurement of household food security and the impact of food aid programs in humanitarian emergencies. CARE and WFP, Nairobi. 2003.

**Supplementary Table 2: Cumulative distribution of serious adverse events among HIV-negative women and HIV unexposed infants by randomized trial arm**

Event Type	Study arm				Total
	SOC	IYCF	WASH	IYCF+WASH	
Miscarriages	28	21	31	44	124
Stillbirths	13	26	22	17	78
Neonatal deaths (<1mo)	23	29	31	32	115
Infant deaths	27	13	19	24	83
Maternal hospitalization	27	32	40	43	142
Infant hospitalization	25	28	20	38	111

Number of women/infants recruited into each arm: 997/1011 (SOC), 1008/1022 (IYCF), 1036/1056 (WASH), and 1121/1138 (IYCF+WASH)

## **APPENDIX 2**

### **SHINE RESEARCH PLAN**





# SHINE

## Sanitation Hygiene Infant Nutrition Efficacy Project

ClinicalTrials.gov identifier NCT01824940

Protocol version 19, October 03, 2016

A handwritten signature in blue ink, appearing to read "Jean Humphrey", is positioned above a horizontal line.

\_\_\_\_\_  
Professor Jean Humphrey, PI

*October 03, 2016*

Date: \_\_\_\_\_

## Summary of Changes from version 18

### Major changes:

- Addition of urinary and faecal metabolomics in a subgroup of infants and their mothers. In a group of 1000 infants (500 with 'good growth', defined as LAZ>-0.5 at 18 months; and 500 with 'poor growth', defined as LAZ<-2 at 18 months) we will send urine samples from the 12 month visit to Dr Jon Swann at Imperial College London to undertake untargeted metabolomics by nuclear magnetic resonance spectroscopy and/or mass spectrometry. From a group of 150 mother-infant pairs (longitudinal samples from 32 weeks and 1 month postpartum for mothers, and 1, 3, 6, 12 and 18 months for infants) we will send stool samples to the University of British Columbia, Vancouver, to undertake untargeted metabolomics by nuclear magnetic resonance spectroscopy. Metabolomics is a powerful technology for assessing metabolic pathways without a priori hypotheses, which will provide unique insights into the pathogenesis of stunting beyond the EED pathway that we are already testing, but methodology is not available in Zimbabwe for this work.
- Addition of a qualitative study of mothers' experiences using the SHINE interventions in which we will purposively sample up to 80 mothers (20/arm) to conduct an in-depth interview. Consent will be obtained using **SHINE Form 76 v1 Mother's Experience with Interventions Consent Form** and the in-depth interview using **SHINE\_ Form 77\_v1\_ Mother's Experience with SHINE Interventions Questionnaire**.
- Addition of observation study to be conducted once on 180 mother-infant pairs. These households will be selected by matching the gender and age of the youngest 90 infants in the WASH arms to 90 infants in the non-WASH arms. The purpose of these visits is to check compliance with WASH and IYCF behaviors, and therefore provide an additional measure of intervention uptake. In addition, a trained observer will conduct a 6-hour structured observation. The researcher will visit the mother and obtain consent using **SHINE\_ Form 78 v1\_Observation Study Consent Form**. After obtaining consent the researcher will observe the household as well as the normal daily routines of the mother using **SHINE\_ Form 79\_v1\_Household and Baby Observation Form** to record specific baby activities and household observations.
- Removal of unscheduled visits. We have removed microbiology testing and **SHINE Form 42v1 Unscheduled Visit Form**.
- Request to change recipient of aflatoxin-lysine analyte of extracted serum samples for measurement of aflatoxin-lysine by mass spectrometry from John Hopkins University USA to University of Maryland College Park,USA.

### Minor Changes:

- Correction to research questions 13, 14 and 15 in the Secondary Specific Aims; these questions will explore relationships between pregnancy exposures and mean birth length, birth weight and gestational age
- Update of the preterm birth substudy, which now includes 'small for gestational age' as an outcome together with preterm birth. This enables us to explore the overlap between SGA and preterm in infants.
- We have updated the methodology regarding the adverse birth outcome substudies to clarify the approach that will be taken for each, particularly regarding selection of cases and controls.
- Addition of infant scores to the OMCI test on **SHINE\_Form63\_v5 24 Month Visit Data Collection Form**
- Clarification that 24 month visits will be conducted even if the infant has moved from the primary SHINE home

- SHINE\_Form 70\_v2\_Neurodevelopment and Body Composition at 24 months Visit Consent has been revised on procedure to now measure the child's leg length distance between the knee and the heel instead of between the knee and the ankle.
- 
- **SHINE\_Form 71\_v3\_Body Composition Data Collection Form** has been updated. mid-thigh circumference has been changed to mid-calf circumference.
- We have updated the current panel of biomarkers being used to assess maternal and infant EED throughout the proposal, and added FUT2 measurement in infant saliva
- We have changed the location for sample export to analyze plasma for measurement of the tryptophan metabolism pathway and citrulline, from Oregon Analytics, USA to Imperial College London, UK.
- We clarified sample sizes for mycotoxin measurement. In summary, aflm1 will be assessed on all enrolled women at baseline and 32-34 weeks gestation and 1600 EED infants at 3, 6, 12 and 18 months. Aflatoxin-lysine and multiple urinary mycotoxins will be assessed in women enrolled in the miscarriage, stillbirth and preterm/SGA substudies. and 1600 EED infants at 6, 12 and 18 months. Clarifications appear on pages 37, 51, 52 and 61.
- We have updated **SHINE\_Form 55\_v2\_Birth Facility Information** to include parity.
- Addition of Dadirai Fundira as a student investigator
- On Supervision roles and responsibilities we have removed Franne Van Der Keilen who left the organization in May 2016.
- We have also removed Dr Mduduzi Mbuya (**PhD**) who also left the organization in September 2016 from Supervision roles and responsibilities and added him as an External Investigator, as he continues to contribute towards SHINE study.

### Summary of Changes from version 17

- Addition of a new information sheet, SHINE\_Form 75v1\_Diarrhea Sample Collection Information Sheet to improve specimen recovery rates in the diarrhea substudy page 60.

### Summary of Changes from version 16

#### Minor Changes:

- Guardian consent will be obtained where a biological mother is not able to consent at the 24 month visit using **SHINE\_Form 72\_v1\_Guardian Neurodevelopment at 24 months Visit Consent**.
- Addition of **SHINE\_Form 73\_v1\_Specimen Storage and Shipment at 24 months Visit** to obtain consent to store and ship samples collected at the 24 month visit, or **SHINE\_Form 74\_v1\_Guardian Specimen Storage and Shipment at 24 months Visit Consent** when obtaining consent from a guardian.
- Clarification that body composition at 24 months will be assessed in the subgroup of children turning 24 months of age from September 2016. Addition of mid-thigh circumference measurement to this visit.
- Addition of **SHINE\_Form 71\_v2\_Body Composition Data Collection Form** to collect data on body composition at 24 months.
- Addition of collagen X as a biomarker of bone growth, measured by ELISA in infants recruited to the EED substudy (page 61).
- AFM1 will now be measured on all enrolled women at baseline and 32 weeks, instead of the original subgroup of 3200 women (page 36), to provide more complete data on short-term aflatoxin exposure during pregnancy.

- Request for shipment of samples to perform rotavirus IgA assays at the University of Vermont, USA, because methodology cannot be established in country.
- Addition of responsibility to the Research Administration and Compliance Manager. (page 82)

## Summary of Changes from version 15

### Major Changes:

- Addition of bioimpedance analysis, skinfold thicknesses and leg length measurement to the 24 month visit, to more fully assess the impact of the randomized interventions on infant body composition, with consent obtained using **SHINE \_Form 70\_v1 Neurodevelopment and Body Composition at 24 months visit Consent Form**. (Table 9, and page 57). Details of the devices are listed in section 11b.

### Minor Changes:

- Sample size for EED substudy updated on page 24 to explain the detectable difference in biomarkers between groups.
- We have updated the list of assays to be conducted on maternal and infant biospecimens to reflect current panels of markers that best define EED and oral vaccine responses. We have therefore removed AGP, IL-6, IL-1b and IGFBP3 from the assay list, and added kynurenine:tryptophan ratio (plus metabolites along tryptophan/IDO1 pathway, AhR agonists and serotonin) and tetanus IgG. Additionally, to gain insights into functional immune responses, we have added whole blood stimulation using pathogen-associated molecular patterns at the 18 and 24 month visits (Table 9).
- In line with the changes in biomarkers above, we are requesting permission to ship plasma samples for all mothers and infants requiring citrulline, kynurenine:tryptophan ratio (plus metabolites along tryptophan/IDO1 pathway, AhR agonists and serotonin) and essential amino acids to Oregon Analytics in USA, where they will be measured by mass spectrometry.
- We have added analysis of enteropathogens by Taqman Array Card on stool samples of infants in the EED substudy. These will be analyzed in conjunction with diarrhoeal specimens using the same technique (at University of Virginia, USA), which will allow us to calculate the attributable fraction of diarrhea caused by each pathogen, accounting for carriage of enteropathogens in EED 'control' specimens.
- Addition of FUT2 as a salivary analyte in women to evaluate maternal capacity to fucosylate human milk oligosaccharides, which is linked to infant growth.
- Clarification in Table 9 that HIV DNA PCR will be conducted on all infants whose mothers are known to be HIV-positive at the 18 and 24 month visit, as stated in the consent form, with results returned to the caregiver.
- We have reduced salivary collection at the 24 month visit from 3 infant saliva samples to one single sample (Table 9).
- We have simplified the assessment of early child development by removing the assessment of behavior and mood using the frustration task (Table 3).
- Addition of **SHINE\_FORM 67\_v 1 Parent Information sheet** listed in the protocol as the information sheet provided to caregivers of children with developmental delay.
- Addition of Dr Joseph Piper as a student investigator

## Summary of Changes from version 14

### Major Changes:

- Recruitment figures have been updated to 5282 women total, to reflect the 10% over-recruitment that was conducted to ensure sufficient power to conduct sensitivity analyses. This was particularly important in the WASH arms because of the failure of the original subcontractor of latrine construction (Oxfam UK) to ensure timely installation of latrines per protocol for the first 730 enrollees.

#### Minor Changes:

- Addition of urinary aflatoxin M1 as a measure of aflatoxin exposure in up to 1000 infants at 12 and 18 months of age.**
- End of allowable window for 24 month visit extended from 110 weeks to 112 weeks
- Update of method used to assess infant behavior and mood following piloting of tool (Table 3)
- Updated data collection form **SHINE\_Form 63 v2\_24 month visit data collection form** to reflect final tests to be administered following piloting.
- Addition of a third infant saliva sample at the 24 month visit in order to monitor stress recovery in children, and removal of maternal saliva sample from the 24 month visit (Table 9). This is reflected in amended consent form **SHINE\_Form 65\_v2 Neurodevelopment at 24 months Consent Form.**
- Submission of a new information sheet (**SHINE\_Form 67\_v1 Parent Information Sheet 24 Month Visit**) that can be given to caregivers of children following Early Child Development assessments.
- Removal of the intensive 24 hour dietary recall for infants in the EED substudy at the 18 month visit (Table 10)
- Updated data collection form **SHINE Form 43v2 24Hr Dietary Recall Form** to address two minor issues observed in 3 questions: a lack of clarity observed in initial interviews and separation of a two part question (Page 58).
- Addition of a two-weekly reminder phone call to mothers of infants in the diarrhea substudy (page 58). This is to improve collection of specimens for infants who develop diarrhea.
- Change to specimen collection schedule at 18 months if mother-infant pairs are visited at long distance, out of the study districts. Where it is not possible to get specimens processed in the hub laboratory the same day, infants will instead have a dried blood spot card stored, and mothers will not have a blood sample collected (Tables 9 and 10).
- Provision of an information sheet (**SHINE\_Form 68\_v1 Specimen and Testing non EED Information Sheet**) for mothers of non-EED babies at the 18 month postnatal visit, and an information sheet (**SHINE\_Form 69\_v1\_Specimen and Testing EED Information Sheet**) for mothers of EED babies to explain the reasons for specimen collection and haemoglobin testing.
- Local anaesthetic cream will be available for infants to reduce the pain associated with venipuncture if the mother wishes (Tables 9, 10).
- Addition of Jaya Chandna as a collaborator (Page 79).

#### Summary of changes from version 13

##### Minor Changes:

- Addition of 70 non SHINE mothers to step 2 of piloting the McArthur Bates language assessment test
- Reduction from 70 to 20 SHINE mothers to step 3 (piloting ALL cognitive tests)

#### Summary of changes from version 12

##### Major Changes

- Addition of three research questions, to assess the impact of the SHINE interventions on early child development. This requires a new visit at 24 mo of age, with additional informed consent taken for this additional visit, which will enable us to more fully evaluate the impact of the randomized interventions on health and growth, by assessing domains of child development that are not captured in current outcome measures. This additional visit allows us to evaluate more completely whether SHINE interventions helped children to ‘thrive’ and harness their full human potential, beyond the measures of illness and linear growth that were already addressed. The new Secondary Aims are stated on page 16 (Aims 16, 17, 18), and the description of the new visit is outlined on page x.
- We are requesting permission to ship samples for all mothers and infants requiring lactulose-mannitol measurement in urine samples to Oregon Analytics in USA.
- Pages 73-77: on advice of the international IRB (Johns Hopkins) and local IRB (MRCZ), we have altered the reporting process for Adverse Events (AE) and Serious Adverse Events (SAE). We will only report AE and SAE within the expedited time-frame if they are judged to be uncertainly, probably or definitely related to the study interventions or procedures. Events that are unlikely related to study interventions or procedures will be reported annually in tables, split by AE/SAE type.

### Minor Changes

- Addition of measurement of alpha-1-antitrypsin and neopterin in maternal serum. Protocol already included measurement in stool. This change was made to fully characterize these biomarkers in women which represent inflammation and gut damage. (pg 50 and Table 3)
- Revision of the section “Developmental Evaluation of the protective play space intervention component” to include participants of the non-WASH (SOC and IYCF) arms of the study. This change was made in order to understand the positive and intentional impact of the WASH intervention as well as any unintended consequences that might result; in addition to the original intent of strengthening the playspace component of the intervention. Accordingly, the exploration has been renamed in the protocol to “Qualitative study to assess the potential effectiveness of the SHINE WASH intervention” on page 45-6.
- Continue recruitment to the Environmental Enteric Dysfunction substudy beyond the original sample size of 1000 HIV-negative mothers and their infants. We plan to enroll all mothers who joined SHINE from 1<sup>st</sup> May 2014 to the end of recruitment. This will ensure at least 150 children per trial arm have longitudinal assessments of EED (allowing for missed samples and loss to follow-up) to allow the substudy to be adequately powered.
- Secondary Aim 8 has been amended to remove “between birth and 6 months of age”
- Table 7 footnote - If women request an HIV test at other study visits to learn their status, we will undertake a rapid test in the home where feasible.
- Table 9 footnote - Anthropometry data and hemoglobin result at 18 months (primary trial endpoints) will be checked carefully by Research Nurse Supervisors at the SHINE hub; in cases where primary endpoints are either missing or outside the stipulated plausibility ranges, a home visit will be conducted to repeat the measurement(s).
- Table 11 footnote – if an infant HIV DNA PCR is indeterminate we will conduct a home visit to give the result to the caregiver, but will request a repeat sample so that we can repeat the test and return the result to the mother to clarify the infant’s HIV status.
- Page 59: If maternal HIV status was not confirmed using samples collected at baseline and/or 32 weeks, HIV testing will be repeated in EED substudy mothers using the maternal sample collected at 1mo postnatal, to ensure final maternal HIV status has been determined, as stated in the main trial consent form.

- Clarification of consent procedure for illiterate women (section 7c).
- Page 78: Updated role of Assistant Director, Field Operations and Research Administration and Compliance Manager

## **Summary of changes from version 11**

### **Major Changes**

- Addition of the measurement of T-2 toxin, zearalenone and ochratoxin A in mothers in the preterm birth substudy and 200 infants.
- Request to ship urine from mothers and infants to University of Muenster, Germany for measurement of fumonisin, deoxynivalenol, T-2 toxin, zearalenone and ochratoxin A (page 32).
- Addition of an exclusion criterion to the EED substudy: infants with a major non-fatal abnormality that is likely to directly affect gut health/function or stature (e.g. neural tube defects, cerebral palsy, Down syndrome). This is because additional procedures may cause undue discomfort or pose additional risk to these infants, and will yield data that are difficult to interpret in view of their underlying medical condition.

### **Minor Changes**

- Addition of measurement of multiple mycotoxins at 6 months and 18 months of age in 200 infants and removal of measurement at 3 months.
- Change of measurement of multiple mycotoxins in the mothers enrolled in the preterm birth substudy instead of 200 mothers in the EED substudy.
- Change to request to ship aflatoxin-lysine analyte to Johns Hopkins University, USA and/or University of Muenster, Germany.
- Amendment to one secondary aim (number 14), to include the question of whether adverse effects of maternal schistosomiasis are mediated through inflammation.
- Addition of a laboratory assay to measure poliovirus IgA in infants enrolled to the EED substudy as a measure of oral vaccine immunogenicity. This will allow us to address the question of whether a WASH intervention improves immune responses to polio vaccine as well as rotavirus vaccine (EED substudy aims 13 and 14 updated).
- Extend outer window of 18 month visit to 130 weeks.

## **Summary of changes from version 10**

### **Major Changes**

- Request to ship aflatoxin-lysine analyte from extracted maternal and infant plasma samples to Johns Hopkins University, USA, for measurement of aflatoxin-lysine by mass spectrometry.
- Addition of one research question to the EED substudy to assess the relationship between EED and aflatoxin exposure using urine and plasma samples.

### **Minor Changes**

- Measurement of urinary aflatoxin M1 in 3200 women instead of 2000 women as an additional measure of aflatoxin exposure that will inform seasonality of exposure in the SHINE districts (page 30).
- We have added a new community brochure (Community Brochure Post Recruitment V1) for distribution in the study area after recruitment had ended.



- We have removed stool collection from main trial infants at 18 months of age (Table 9), in line with our decision not to test for soil-transmitted helminthes at 18 months of age. Infants enrolled to the EED substudy will continue to have stool collected at 18 months of age (Table 10).
- We have delayed enrollment into the diarrhea sample collection substudy until 1<sup>st</sup> June 2015, to provide more time to prepare for this substudy in the field. We have also changed the timing of consent from the 1 month postnatal visit to the 3 month postnatal visit, which is a less busy visit and provides more time to undertake the consenting process.
- We have clarified the HIV testing procedure for women and infants in the main trial at 18 months postnatal (Table 9 footnotes): if the mother is found to be HIV-positive, she will have a CD4 count measured and the infant blood will be used to test for HIV by DNA PCR and to measure CD4 count; results will be returned to mothers by home visit. For HIV-exposed infants in the EED substudy, results of the HIV DNA PCR will be returned to the mother by home visit (Table 10, footnote).

## Summary of changes from version 9

### Minor Change

- On page 52 we have amended Diarrhoea Sub-study Visit form to version 3 and now reads....SHINE\_Form 51v3 Diarrhoea Sub-study Visit on page 53.
- On Page 53 we have corrected the word Welfare to Care.

## Summary of changes from version 8

### Major changes

- We have removed testing of infant stool for soil-transmitted helminths (STH) at 18 months of age. This is because the 2010 National Helminthiasis Survey showed very low prevalence of STH in Shurugwi and Chirumanzu districts (1.5% and 0% among school-age children, respectively), and we would expect the prevalence to be even lower in 18 month old children. Because the laboratory methods for helminth detection are not highly sensitive, we have decided not to test stool samples routinely at 18 months. Stools are being stored at -80C and could later be used to diagnose STH by PCR if this was necessary for later analyses. We have therefore removed details of the Kato Katz test from the Diagnostic Tests section. We have added an explanation of how we will inform the study participants: that we will not test their children for helminthes because a large survey conducted by the Ministry of Health has demonstrated this is not a problem among children in Chirumanzu and Shurugwi.

### Minor changes

- We have changed the sample type collected at 18 months for infants in the main trial, from one EDTA and one serum gel clot tube, to two EDTA tubes. This is to simplify procedures in the field and the laboratory, because all analytes can be measured on plasma. The volume of blood collected has not changed, only the blood tube.
- We have updated enrolment procedures for the EED substudy so that women who miss the 32 week gestation visit can provide consent to join the EED substudy at the earliest opportunity thereafter.
- We have clarified in Tables 9 and 10 that infants enrolled in the EED substudy should have specimens collected according to the EED substudy schedule (Table 10) and not the main trial specimen collection schedule (Table 9).

- We have clarified that diarrhoeal specimens will only be collected from infants who are enrolled to the EED substudy from March 2015 onwards, and that we will ask mothers to inform us if their infant has blood or mucus in the stool, in addition to watery diarrhoea. Data will be collected at diarrhoeal specimen collection visits using **SHINE\_Form 51v2 Diarrhoea Sub-Study Visit**.
- We have updated Tables 7, 9 and 10 to state in the Footnote that missing biological specimens will be collected as soon as possible after a missed visit or failed collection attempt, and that this may occur outside the window of the original visit. This is to minimize missing data in the trial.
- We have updated the turnaround time of HIV testing for infants. The result will be provided to the mother within 4 weeks of collecting the specimen, because of the logistics of transporting dried blood spot specimens from the field to Harare, undertaking HIV DNA PCR testing and returning the result to the mother.
- We are requesting permission to provide women enrolled into the SOC and IYCF arms a certificate at the 18 month visit which pledges that she will have a latrine constructed at the end of the trial. This is to avoid any doubt on the part of the mother that a latrine will be constructed by SHINE.
- We have added an additional section on Adverse Event reporting to the Johns Hopkins University IRB in line with their reporting guidelines.

## Summary of changes from version 7 to version 8

### Major changes

- Addition of two research questions to the EED substudy to assess the relationship between EED and rotavirus vaccine immunogenicity (aim 13), and the impact of WASH interventions on rotavirus immunogenicity (aim 14), using plasma samples already being collected. This aim has been added to leverage the roll-out of rotavirus vaccination within the EPI programme in Zimbabwe. Immunogenicity is defined in Table 3 and added to assays to be undertaken on infant plasma (page 48).
- Addition of microbiological sampling to the unscheduled visit for 800 women (page 45) to assess the impact of WASH interventions on environmental contamination.
- Addition of diarrhoeal stool specimen collection for infants enrolled in the EED substudy (Table 10, and page 48), to assess the pathogens causing diarrhoea in this trial by TaqMan array card PCR, with consent obtained using SHINE\_Form 50v1 Diarrhoea Sample Collection Consent Form and data collected using SHINE\_Form 51v1 Diarrhoea Data Collection Form.
- Request to analyze 50 maternal plasma samples for aflatoxin-lysine by mass spectrometry at Johns Hopkins University, USA.
- Addition of a Qualitative Study of Intervention Nurse evaluation of VHW performance Consent form using SHINE Form\_52v1 and SHINE Form\_53v1, Intervention Nurse's perception of VHW performance Interview Guide.
- Addition of SHINE\_Forms 57, 58, 59, 60\_v1\_Reconsent Form for participants who have withdrawn but wish to resume their participation in the study.

### Minor changes

- Update of Secondary specific aim no 9, to remove anemia at 30-34 weeks gestation (since hemoglobin is not being assessed at this timepoint) but to add mycotoxin status.
- Addition of SHINE\_Form 54v1\_End of Pregnancy Catch up Form to Table 5.

- Addition of SHINE\_Form 55 Birth Facility Information to collect data on newborn babies from health facility registers (this was previously included in the End of Pregnancy form, but has been taken out to create a new, updated form).
- Addition of SHINE\_Form 56v1\_Baseline Catch up Form to Table 5.
- Change of consent procedure for unscheduled observations; we have eliminated SHINE\_Form \_41v1\_ Unscheduled Consent Form; instead, consent for unscheduled observations and microbiological sampling will be obtained at trial enrolment, or using SHINE\_Form 46-49v1\_Consent Form Addendum.
- Update of exit procedures from SHINE trial, using updated SHINE\_Form 24\_v3\_Exit form
- Updating of HIV testing procedures for women in Table 7. In addition to the rapid test conducted in the woman's home, the same sample will be tested for HIV using a rapid test algorithm in the laboratory, to confirm the result obtained in the home.
- Correction of Table 9 to include paternal height, since this measurement will be taken once at any point during the trial, including during the postnatal period.
- Change to the selection criteria for EED substudy; instead of selecting a subgroup of maternal-infant dyads to join the study, all mothers will be invited to join at the 32 week gestation visit until 1000 HIV-negative mother-infant dyads and all HIV-positive mother-infant dyads have been recruited.
- Addition of HIV DNA PCR testing for infants born to HIV-positive mothers at 1mo of age to enable timely diagnosis of HIV infection; CD4 counts will be measured in all HIV-exposed infants. Dried blood spot cards will be made in the laboratory, and not in the field.
- Addition to pre-LM test single urine sample collection from infants in EED substudy (Table 10), as per SOP, prior to ingestion of lactulose-mannitol solution.
- Addition of aflatoxin M1 (AFM1) and creatinine measurement to urine samples from the first 2000 women in the trial, as an additional measure of aflatoxin exposure that will inform seasonality of exposure in the SHINE districts (Table 3, and page 28).
- Update of Loss to Follow Up section to state that intervention delivery will resume if women return to their primary home after moving away.
- Updates to Supervision Roles and Responsibilities (section 15).

## Summary of Changes from version 7

### Major Changes

- Change in inclusion/exclusion criteria, as below:

*Inclusion:* Pregnant women residing in randomized clusters within the study districts, whose pregnancy is confirmed by a urine pregnancy test.

*Exclusion:*

- Women residing in the study districts who become pregnant during the enrollment period but do not consent to join the trial
- Women who reside in urban areas of these two districts
- Women who are resident as employees at a homestead or who are living on rented premises

## Summary of changes from version 6

### Major changes

- Addition of new substudy, 'Risk Factors for Neonatal Death Substudy'

- Change of sample collection protocol in Environmental Enteric Dysfunction substudy, substituting PAXGene RNA Blood Tubes for serum tubes. Tubes will be frozen at -80C for subsequent analysis of gene expression profiles in stunted infants.
- Addition of DNA collection from all mothers and infants, on advice of the DSMB, for future studies of genetic determinants of infant growth and health. The Specimen Storage and Shipment Consent Form 9v4) has been updated to request consent to extract DNA from existing samples from women. Addition of cell pellet storage from all infants at 18 month blood draw to enable DNA extraction if consent is provided. The section on the biospecimen repository (section 17) has been updated to provide detail about DNA and RNA storage.
- Addition of consent form addenda (SHINE\_Form 46-49v1\_Consent Form Addendum, April 1, 2014) for already enrolled participants that covers the addition of, and informed consent for, DNA collection from mother and baby (above), a request for birth notification, clarification on the implications of moving on study participation, and provision of a small incentive gift to the baby in line with cultural norms (Makorokoto).

### Minor Changes

- Change of terminology from Environmental Enteropathy (EE) to Environmental Enteric Dysfunction (EED)
- Change from 'maternal HIV status' to 'maternal/infant HIV status' in all analyses of secondary specific aims
- Addition of a small non-monetary gift for all postnatal visits
- Addition of a solar charger for women enrolling into the EED substudy so that they can charge the cellphone provided to improve communication regarding the morbidity diary.
- Change to windows for each study visit; extending the allowable range of data collection to the start of the next scheduled visit to minimize missed visits, and correcting the weeks of intervention delivery (computed using a conversion factor of 4.33) in line with the calendar months of child's age (Table 5).
- Addition of a qualitative exploration of mothers' experiences in receiving the SHINE interventions.
- Addition of structured observations and interviews designed to strengthen the delivery and uptake of the "Protection of a Developing Child" module (W3) among households in the two WASH intervention arms; with separate consent form (SHINE Form 36v1 developmental evaluation consent form) and data collection forms (SHINE Form 37 v1 play space developmental evaluation Visit 1x) and (SHINE form 38 v1 play space developmental evaluation Visit 2).
- Addition of paternal height to data collection activities (Table 7) with separate consent form (SHINE Form 40\_v1 Parental Height Consent form).
- Addition of aflatoxin M1 measurement in urine collected from 1500 mothers at baseline visit.
- Addition of unscheduled data collection visits for 800 women on one occasion, to provide an additional measure of the uptake of interventions. A separate consent form will be used for these unscheduled visits (SHINE Form \_41v1\_ Unscheduled Consent Form) and data collected using SHINE Form 42v1\_ Unscheduled Visit Form
- Extension of enrolment period for HIV-exposed infants to the EED Substudy to include those who have been born prior to launch of the EED substudy to ensure HIV status can be ascertained on these infants.
- Addition of intensive 24 hr dietary recall (SHINE Form 43v 1 Dietary Recall Form) for 50 randomly selected infants per arm in the EED substudy (Table 10)
- Additional details provided about the infant morbidity diary for infants in the EED substudy. Details provided of a pilot evaluation for the first 40 infants in the EED substudy to determine the best means of support for mothers in diary completion, comparing phone calls, SMS and home visits.

- Addition of two new forms to collect Verbal Autopsy data for mothers or infants who die beyond the End of Pregnancy visit (SHINE Form 35 v1 Maternal Verbal Autopsy and SHINE Form 45v1 Infant Verbal Autopsy, respectively).
- Clarification regarding interventions provided to women who move from their original homes (Loss to Follow Up section) and change from making only 2 attempts at each research visit to enabling more visits to be made, in line with the extended windows for each visit.
- Addition of plasma citrulline as an additional biomarker of EED (Table 3).
- Change of SHINE Community Brochure v4 on contact numbers and inclusion of LMS given to mothers.
- Use of a new **SHINE EED Community Brochure v1** to sensitize study participants and the community about the EED substudy.
- Addition of a definition of 'guardian' in situations where the mother dies.
- Addition of more detail about the HemoCue 301 haemoglobinometer specifications.
- Addition of a Monitoring Plan for SHINE (see section 12d)
- Updating of definitions of malnutrition for infants (Table 14)
- Addition of plans to minimize group harm within the SHINE trial, to sections 8 (e) and 17(h).
- Clarification of procedures for consent and for destruction of audiotape recordings in the 'Qualitative study of mothers' experiences in receiving the SHINE interventions' and 'Developmental Evaluation of the protective play space intervention component' interviews.
- 

## Summary of changes from Version 5

### Major Changes

- Increase recruitment by identifying eligible women at Antenatal Clinics and increasing gestational age inclusion criteria from <18 weeks to <24 weeks gestation using SHINE Form 27 v1 ANC Surveillance Form.

### Minor Changes

- Minor changes to forms to be compliant with ANC recruitment:
  - Addition of SHINE Form 27\_v1\_ANC Surveillance Form
  - Change to timing of baseline visit from 10 - 20 weeks, to 10- 26 weeks using SHINE Form13\_v4\_Baseline Survey Form
  - Change to Consent forms 2,3,4,5 to reflect increase in gestational age eligibility
- To correct research questions for EE Substudy to include both gestational time points.
- Add Module BV to Postnatal Forms SHINE 20\_v2\_ 3 Month Visit which was mistakenly omitted in \_v1.
- To add a consent form for the guardian to use in the case that a mother dies and the baby survives using SHINE Form 28v1 Guardian Consent
- Changed Ministry of Health and Child Welfare (MoHCW) to Ministry of Health and Child Care (MOHCC), their recently altered name.
- To reduce transportation costs in collecting morbidity data from EE substudy mothers by providing these mothers with a cellphone and airtime

## Summary of changes from Version 4

## Major changes

- Addition of new substudy (Risk Factors for Miscarriage)
- Change to inclusion criteria: gestational age eligibility increased from <14 weeks to < 18 weeks
- Removal of exclusion criterion: infants with congenital malformations
- Change to timing of baseline visit from 10-16 weeks, to 10-20 weeks
- Addition of one research question to the environmental enteropathy substudy (specific aim 8)
- Addition of 4 new standard of care modules (S1-S4) to the intervention schedule (Table 6)
- Addition of lactulose mannitol test for infants in the environmental enteropathy substudy at 3 months of age
- Change to adverse event and serious adverse event reporting processes

## Minor changes

- Changes to the sample size calculation for the EE sub-study
- Updated definition of depression in mothers and environmental enteropathy in women and infants (Table 3)
- Updated timepoints in Table 5
- Addition of SHINE\_Form25\_v1 for collection of home birth data
- Addition of CD4 counts for HIV positive mothers at baseline and at 30-34 weeks gestation (Table 7) and for HIV-infected infants at 3, 6, 12, and 18 months gestation (Table 10)
- Addition of cytokine measurement on saliva samples.
- Table 9: Updated postnatal data collection, addition of birth visit for home births, and stool collection from all infants at 18 months
- Change in timing of informed consent process for the EE sub-study to 32 week visit
- Reduction in number of morbidity diary visits for women in the EE substudy and updated total number of visits for mother-child pairs in the study
- Clarification of loss to follow-up definitions
- Change in Data Safety and Monitoring Board membership from Prof James Hakim to Dr. Tariro Makadzange
- Updated title of Mr Robert Ntozini to Associate Director IT/Data/Statistics.

## Summary of changes from Version 3

### Major changes

- Addition of two research questions (specific aims 10 and 11) in the environmental enteropathy substudy
- Clarification of inclusion criteria: women must be within first 14 gestational weeks, live within randomized clusters and be permanent residents in the study districts
- Change in timing of lactulose mannitol test from 5hr to 2hr urine collection
- Increase in blood volume collected from women, from 12ml to 15ml; reduction in blood volume collected from infants, from 5.9ml to 5.4ml
- Addition of 1 month postnatal collection of blood and stool from mother-infant pairs enrolled in the environmental enteropathy substudy
- Removal of 9 month and 15 month postnatal visits

- Change to adverse event and serious adverse event reporting processes

**Minor changes**

- Updated map of clusters
- Additional study outcome definitions added to Table in section 4 (Measurements and Definitions)
- Update to table of visits and table of intervention modules and addition of allowable windows of timing around each visit.
- 7 month antenatal visit renamed 32-week gestation visit
- The Table on pages 26-28 has been updated to reflect a few minor changes to the data collection schedule.
- Minor updates to Research and Implementation table
- Primary care nurses renamed throughout protocol as SHINE Intervention Nurses (INs) or SHINE Data Collectors (DCs); senior nurses renamed as Research Nurse Supervisors, and data managers as Field Data Supervisors.
- Updated sample sizes for mycotoxin analyses
- Details of methodology for microbiota analysis added
- Addition of hepatitis A serology on infant 18 month blood samples
- Addition of REG-1 ELISA assay to be undertaken on stool samples
- Addition of postnatal forms to protocol: SHINE\_ Form 20\_v1\_3 months visits; SHINE\_ Form 21\_v1\_6 months visits; SHINE\_ Form 22\_v1\_12 months visits; SHINE\_ Form 23\_v1\_18 months visits
- Clarification of definitions of loss to follow-up, and addition of an exit form (SHINE\_ Form 24\_v1\_ Exit form)
- Addition of membership of the DSMB.
- Change from use of cell stabilized tubes to collection of whole lysed blood samples for analysis of PBMCs
- Reference list updated.

**Summary of changes from Version 2****Minor changes**

- Addition of justification for HIV testing
- Addition of maternal blood collection to table of antenatal visits
- Change in title from “SHINE Nurse” to “SHINE Nurse Data Collector (DC)”

**Summary of changes from Version 1****Major changes**

- Addition of infant and maternal stool collection and infant blood collection at 1 month postnatal visit for mother-infant pairs in environmental enteropathy substudy
- Addition of adverse event and serious adverse event reporting system

**Minor changes**

- Addition of blood volumes to be collected



- Addition of statement that there are no plans for genetic testing on samples
- Addition of intervention-specific brochures to be distributed by Village Health Workers
- Clarification in table of interventions that latrine will be provided at the end of the study to women in the standard care arm
- Addition of insulin-like growth factor binding protein 3 (IGFBP3) measurement on blood samples, and intestinal fatty acid binding protein on urine
- Mycotoxin exposure defined and method of assessment added
- Provision of a calendar to enrolled women to record important dates
- Change in system of notification of eligible women by Village Health Workers from paper form to cellphone SMS
- Addition of mycotoxin exposure assessment at 7 month gestation visit and change in sample size and timepoints for mycotoxin assessment
- Change in screening procedure for women who report vaginal bleeding: consent visit delayed by 2 weeks and women referred to clinic
- Change in stool collection procedure to delay collection for one week after ingestion of lactulose mannitol solution
- Collection of pre-lactulose:mannitol urine sample for urinalysis and microscopy
- Change in clinic referral criteria for anemic women from  $<7$  g/dL to  $<10$  g/dL
- Addition of assessment of end of pregnancy outcome at 1 month postnatal visit
- Change of location of manufacture of lactulose-mannitol solution from local pharmaceutical firm to Zvitambo clean room
- Addition of detail regarding flow cytometry assessments being undertaken
- Addition of questions on maternal health and wellbeing on follow up data collection forms
- Volume of infant blood draw added to EE substudy data collection table
- Definition of loss to follow-up added
- Clarification of HIV testing algorithm and addition of HIV DNA PCR for discordant HIV rapid test results
- Addition of postdoctoral fellows in the investigator list

PI: Jean H Humphrey

**Study Title:** SHINE –Sanitation Hygiene Infant Nutrition Efficacy Project

IRB#4205

**PI Version Number/Date:** Version 19, October 3, 2016

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## Phase IV. Clinical Trial Research Plan

We propose to conduct a 2x2 factorial, cluster-randomized, community-based trial in two rural districts of Zimbabwe where HIV prevalence in pregnant women is approximately 15%. Nested within this large trial are four sub-studies each of which will address research questions that are complementary to the main trial. We have organized the sections of the Research Plan by study (main trial and sub-studies). The sub-studies titles are:

- Environmental Enteric Dysfunction (EED) Sub-Study.
- Risk Factors for Pre-Term Birth Sub-Study.
- Risk Factors for Stillbirth Sub-Study.
- Risk Factors for Miscarriage Sub-Study

**NOTE: This trial is Phase 4 of a multi-phased project. During Phases 1, 2, and 3 we conducted surveys to determine baseline water and sanitation coverage in the study area, surveys and formative research to develop and pilot test the interventions, and implemented blanket interventions across the entire study area [strengthening of the Village Health Worker system and implementation of the WHO 2010 Prevention of Mother-to-Child Transmission (PMTCT) Guidance]. All previous phases were reviewed and approved under MRCZ/A/1534.**

### 1. Research Questions

#### Main Trial

##### **Primary Specific Aims:**

1. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among **HIV negative** women and continued through 18 months post partum, on **stunting** of rural Zimbabwean infants at 18 months of age?
2. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among **HIV negative** women and continued through 18 months post partum, on **anemia** of rural Zimbabwean infants at 18 months of age?

##### **Secondary Specific Aims:**

1. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among **HIV positive** women and continued through 18 months post partum, on **stunting** of rural Zimbabwean infants at 18 months of age?
2. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among **HIV positive** women and continued through 18 months post partum, on **anemia** of rural Zimbabwean infants at 18 months of age?
3. What is the effect of the IYCF intervention on uptake of improved infant feeding practices by maternal/infant HIV status, specifically:

- a. Infant diet quality as assessed by WHO IYCF indicators (WHO 2010)
  - b. Infant nutrient intake from complementary foods assessed by 24 hour dietary recall
  - c. Appropriate use of Nutributter from 6 to 18 months
4. What is the effect of the WASH intervention on the 5 key behaviors it promotes by maternal/infant HIV status?
  - a. Proper disposal of animal and human feces
  - b. Handwashing with soap (HWWS) after fecal contact
  - c. Point-of-use chlorination of drinking water
  - d. Protecting children from ingestion of dirt and feces
  - e. Feeding baby freshly prepared foods, or reheating leftover food
5. For each randomized intervention (IYCF and WASH), is the impact on **stunting** of children at 18 months of age modified by the following factors in addition to maternal/infant HIV status?:
  - a. Household and community socioeconomic and demographic characteristics. For example, maternal age, size of the household, socioeconomic status, etc.
  - b. Village Health Worker (VHW) performance assessed by degree of transfer of correct knowledge to participating mothers and the quality of VHW interpersonal approach
  - c. Maternal capacities (1) social support for mothering or the mother's social networks, (2) access to and control of resources, (3) depression and stress, (4) roles, priorities and time, (5) perceived physical health, and (6) mothering self-efficacy
6. For each randomized intervention (IYCF and WASH), is the impact on **anemia** at 18 months of age modified by the following factors in addition to maternal/infant HIV status?
  - a. Household and community socioeconomic and demographic characteristics
  - b. Village Health Worker (VHW) performance assessed by degree of transfer of correct knowledge to participating mothers and the quality of VHW interpersonal approach
  - c. Maternal capacities (1) social support for mothering or the mother's social networks, (2) access to and control of resources, (3) depression and stress (4) roles, priorities and time, (5) perceived physical health, and (6) mothering self-efficacy
7. What is the effect of the WASH intervention on diarrhea prevalence among infants 0-18 months of age? Is this different among children born to HIV positive and negative mothers?
8. What is the prevalence of exclusive breastfeeding among all infants enrolled in the trial by maternal/infant HIV status?
9. What is the prevalence of anemia among pregnant women at 10-26 weeks by HIV, mycotoxin and schistosomiasis status?
10. What is the prevalence of schistosomiasis among pregnant women living in areas of high prevalence among school-aged children?
11. What is the prevalence of mycotoxin exposure among pregnant women?

12. What is the prevalence and severity of EED among pregnant women at 10-26 weeks and 30-34 weeks gestation by maternal HIV and mycotoxin status, as assessed by:

Indicators of  
Environmental  
Enteric  
Dysfunction  
pathway

- a. Maternal gut inflammation
- b. Maternal gut permeability
- c. Maternal gut microbial translocation
- d. Maternal systemic immune activation
- e. Maternal growth hormone axis

13. What is the association between maternal EED and infant birth outcomes, mean birth weight, mean birth length, mean gestational age at delivery by maternal/infant HIV status)?
14. What is the association between maternal schistosomiasis and infant birth outcomes (mean birth weight, mean birth length, mean gestational age at delivery by maternal/infant HIV status)), and are these associations mediated through inflammation?
15. What is the association between maternal mycotoxin exposure and infant birth outcomes (mean birth weight, mean birth length, mean gestational age at delivery by maternal/infant HIV status)?
16. What is the association between EED and early child development at 24 months of age, stratified by maternal HIV status?
17. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among **HIV negative** women and continued through 18 months post partum, on **early child development** of rural Zimbabwean infants at 24 months of age?
18. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among **HIV positive** women and continued through 18 months post partum, on **early child development** of rural Zimbabwean infants at 24 months of age?

## Environmental Enteric Dysfunction (EED) Sub-study

The purpose of the EED sub-study is to examine hypothesized causal pathways of each intervention, stratified by maternal/infant HIV status:

### Primary Specific Aims:

1. What are the effects of the WASH intervention on each of the following domains of the hypothesized biomedical causal pathway between poor sanitation/hygiene and child **stunting** at 1, 3, 6, 12, and 18 months of age?

Indicators of  
Environmental  
Enteric  
Dysfunction  
pathway

- a. Infant gut inflammation
- b. Infant gut permeability
- c. Infant gut microbial translocation

- d. Infant systemic immune activation
- e. Infant growth hormone axis

2. For the domains listed in #1, what is the association between the variability in each upstream domain with that of the downstream domains (upstream=a and downstream=e along a continuum from a to e) among all children and stratified by intervention group?
3. What is the relative importance of EED vs. diarrhea in mediating the effects of the WASH intervention on stunting?
4. What are the effects of the WASH intervention on each of the following domains of the hypothesized biomedical causal pathway between poor sanitation/hygiene and child anemia at 1, 3, 6, 12, and 18 months of age?

Indicators of  
Environmental  
Enteric  
Dysfunction  
pathway

- a. Infant gut inflammation
  - b. Infant gut permeability
  - c. Infant gut microbial translocation
  - d. Infant systemic immune activation
  - e. Infant hepcidin production as assessed by plasma hepcidin levels
5. Are the associations observed in specific aims 1-4 above modified by HIV status of the mother/infant?
  6. What is the effect of the WASH intervention on diarrhea incidence and duration among infants 0-18 months of age, stratified by maternal/infant HIV status?
  7. What are the associations between inflammatory markers (serum ,TNF- $\alpha$ , , CRP, sCD14 and AGP), iron status and anemia among pregnant women at 10-26 weeks and 30-34 weeks gestation according to their HIV status?
  8. What are the associations between maternal and infant EED, and between maternal EED and infant postnatal stunting?
  9. Is EED more severe among infants who are mixed fed compared to infants who are exclusively or predominantly breast fed, stratified by maternal/infant HIV status?
  10. What is the prevalence of mycotoxin exposure among infants between birth and 18 months, and what is the association between mycotoxin exposure, EED and stunting between birth and 18 months?
  11. What is the impact of the maternal microbiota, infant breastfeeding practices and the WASH intervention on early (0-6 months) intestinal microbiota development and on infant growth between 0 to 6 months?
  12. What is the impact of the early microbiota, the IYCF intervention and the WASH intervention on late intestinal microbiota development and on infant growth between 6-18 months?
  13. What is the association between infant EED and infant rotavirus vaccine and polio vaccine immunogenicity?



14. What are the effects of the WASH intervention on infant rotavirus vaccine and polio vaccine immunogenicity?
15. What are the associations between aflatoxin exposure and maternal EED among pregnant women at 10-26 weeks gestation according to their HIV status?

## **Risk Factors for Preterm Birth and Small for Gestational Age (SGA) Sub-study**

### ***Primary Specific Aims:***

1. What is the association between maternal EED and preterm/SGA\* stratified by maternal/infant HIV status?
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and preterm/SGA\* stratified by maternal/infant HIV status?
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and preterm/SGA\* stratified by maternal/infant HIV status?
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and preterm/SGA\* stratified by maternal/infant HIV status?

\*Four infant groups will be defined based on gestational age and weight (categorized as small-for-gestational age (SGA) or appropriate-for-gestational age (AGA)): preterm/AGA; preterm/SGA; term/SGA and term/AGA (reference group). We will also explore associations between each exposure and stunted-for-gestational age (birth length <10th centile) to evaluate impact on birth length as well as birth weight.

## **Risk Factors for Stillbirth Sub-study**

### ***Primary Specific Aims:***

1. What is the association between maternal EED and stillbirth stratified by HIV status?
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?

## **Risk Factors for Miscarriage Sub-study**

### ***Primary Specific Aims:***

1. What is the incidence of miscarriage, and what are the epidemiological exposures associated with miscarriage in rural Zimbabwean women?
2. What is the association between maternal EED and miscarriage stratified by HIV status?
3. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?
4. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?
5. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?

## **Risk Factors for Neonatal Death Sub-study**

### ***Primary Specific Aims:***

1. What is the association between maternal EED and neonatal death stratified by HIV status?
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?

## **2. Background and Rationale**

Child undernutrition remains a large problem in developing countries with substantial adverse sequelae (Black 2008, Victora 2008). It develops during the first two years of life, when mean weight-for-age and length-for-age Z-scores (WAZ and LAZ, respectively) of children living in Africa and Asia plunge to about –2.0 followed by little or no recovery (Victora 2010).

Under the plausible assumption that children grow poorly because they don't eat enough of the right foods, enormous research effort has focused on identifying dietary solutions. Numerous studies have tested a myriad of nutrient-dense foods and supplements, nutrition education interventions, and infant feeding behavioral-change strategies. A recent review of 38 of the best of these studies revealed that most (but not all) achieved statistically significant growth effects: compared to controls, intervention children gained 0–760 g more weight (0.0 – 0.76 WAZ) and grew 0–1.7 cm taller (0.0 – 0.64 LAZ) by 12 to 24 months (Dewey 2008). Enthusiasm is tempered, however, by the realization that the growth effect

of even the most successful of these studies ( $\sim +0.7$  Z) is equivalent to about one-third of the average deficit of Asian and African children ( $\sim -2.0$  Z).

Prevalent diarrhea has also been implicated. In a pooled analysis of nine studies that together collected diarrhea and growth data on 1393 children, the odds of stunting at 24 mo increased multiplicatively by 2.5% per episode of diarrhea, and 25% of all stunting among 24-mo-old-children was attributable to having five or more episodes of diarrhea in the first two years of life (Checkley 2008). However, other authors have contended that the effect of diarrhea on permanent stunting is small because children grow at “catch-up” velocities between illness episodes (Briend 1990). Hence, the relative contribution of diarrhea to undernutrition, and consequently, the potential impact that diarrhea control programs (i.e., sanitation/hygiene interventions) could have on growth has been unresolved. The recent *Lancet* Undernutrition Series estimated that sanitation/hygiene interventions implemented with 99% coverage would reduce diarrhea incidence by 30%, which would in turn reduce prevalent stunting by a modest 2.4% (Bhutta 2008).

This study will test the hypothesis that a major cause of child undernutrition is Tropical or Environmental Enteropathy, more recently renamed Environmental Enteric Dysfunction (Keusch 2013) a **subclinical** condition of the small intestine caused by poor environmental sanitation and characterized by villous atrophy, crypt hyperplasia, increased permeability, inflammatory cell infiltrate, and modest malabsorption.<sup>8</sup> Furthermore, the study will test the hypothesis that the primary pathway from sanitation/hygiene to child undernutrition is via EED rather than diarrhea. If this is true, previous analyses may have substantially underestimated the effect of sanitation/hygiene on growth because the effect was modeled entirely through diarrhea, and sanitation/hygiene interventions may have been undervalued because they have been appraised primarily for their impact on diarrhea.

### 3. Participants

#### Main Trial

##### ***Study Population***

Study participants will be women who are rural residents of Chirumanzu or Shurugwi districts in Zimbabwe and who become pregnant during the enrollment period of the trial and are identified and consent to participation before  $\sim 24$  weeks of gestation, and their live born infants. A total of 4800 women will be enrolled.

All Village Health Workers working in the two districts will be approached to provide their informed consent for assessment of their work capacity, performance and perceptions about the motivational and supervisory characteristics of their work. A total of 430 VHWs will be enrolled.

##### ***Inclusion/Exclusion Criteria***

*Inclusion:* Pregnant women residing in randomized clusters within the study districts, whose pregnancy is confirmed by a urine pregnancy test.

*Exclusion:*

- Women residing in the study districts who become pregnant during the enrollment period but do not consent to join the trial.
- Women who reside in urban areas of these two districts
- Women who are resident as employees at a homestead or who are living on rented premises

### **Sample Size**

A total sample size of 4800 pregnant women will be enrolled, 1200 in each of 4 treatment arms. The primary outcomes are growth and anemia of children at 18 months of age, stratified by maternal HIV status. The main trial inference will be based on outcomes of infants of HIV-negative women, of whom we expect at least 4080 (more likely, 4200) based on a 15% HIV-positive rate among pregnant women. Allowing for 20% loss of evaluable infants at 18 months (this includes pregnancy losses, infant mortality and loss to follow up), we expect about 816 measurements in each of the four study arms. With Type I error of 5%, and power of 90%, and control group stunting (height-for-age Z score <-2) of 30%, and the assumption that the effects of the two interventions will be additive, we will be able to detect a reduction of about 8 percentage points, i.e. from 30% to 22.0%, for either of the interventions (Hayes, 1999). Among the expected 720 pregnancies in HIV positive women, we expect a minimum of 25% loss through fetal loss, etc. resulting in 540 total children at 18 months of age, or 135 per treatment group.

This is based on an assumed coefficient of variation of the true proportions of 0.43, and an effective loss of 33% of sample size due to cluster size variability, which corresponds to a design effect of 2.5. Note that the largest within-unit correlation reported by Katz for stunting in several countries was only 2.6 (Katz, 1995). Using the design effect of 2.5, we can detect a shift of 0.2 weight-for-age and length-for-age Z-scores, assuming a within-randomized-cluster variance of 1.25.

*Hemoglobin:* Assuming a SD at 18 months of 12.8 g/L, we will be able to detect a shift of 2.6 g/L.

**Sample size update:** The final sample size will be 5282 enrolled women, reflecting 10% over-recruitment to provide sufficient power to conduct sensitivity analyses. This is particularly important for the WASH arms, because the initial subcontractor (Oxfam UK) failed to ensure timely installation of latrines and Tippy Taps for the first 730 women enrolled in WASH arms.

**Identifiers** Names, addresses, and antenatal booking number will be collected on all participants at baseline and also recorded on all subsequent study instruments to confirm identity. Household GPS location will be collected at one visit. Each mother-infant pair will be given a unique study ID which will be used to identify all data collected from the pair and will also be recorded on all study instruments. When the data are transferred to the study office in Harare, all personal identifiers except the Study ID will be stripped from the files before adding the data to the database. A list matching personal identifiers and unique identifiers will be kept in a password-protected file on a computer in a locked office which will only be accessible by research staff.

## **Environmental Enteric Dysfunction Sub-study**

### **Study Population**

The study population for this sub-study will consist of two groups:

- a. HIV-negative mothers and their infants:

*Inclusion:* women enrolled in the trial from May 1st 2014 onwards who test HIV-negative at their last HIV test done during antenatal care and who consent into the sub-study at the 32 week gestation visit, or as soon as possible thereafter.

*Exclusion:* 1. HIV-negative women enrolled in the trial prior to May 1st 2014, and

women who refuse to learn their HIV test result. 2. HIV-negative women enrolled in the trial after May 1, 2014 whose child has a major non-fatal abnormality that is likely to directly affect gut health/function or stature (e.g. neural tube defects, cerebral palsy, Down syndrome)

- b. All enrolled HIV-positive mothers (~600) and their infants.

*Inclusion:* women enrolled in the trial who test HIV-positive at any time

*Exclusion:* 1. HIV-negative women, and women who refuse to learn their HIV test result. 2. HIV-positive women enrolled in the trial after May 1, 2014 whose child has a major non-fatal abnormality that is likely to directly affect gut health/function or stature (excluding HIV infection) (e.g. neural tube defects, cerebral palsy, Down syndrome).

### **Sample Size**

This original sample size was decided based on calculations of lactulose:mannitol ratio, endotoxin core antibody and total IgG. We used conservative estimates of standard deviations of 0.3 for the lactulose:mannitol, 101 MUx10<sup>3</sup>/L for endotoxin core antibody, 2.78 g/L for total IgG, and assumed  $\beta=0.20$ ,  $\alpha=0.05$ , and two-sided tests. With 150 infants per group we will be able to detect effect sizes ranging from 35% to 40% of one standard deviation for each of these indicators when comparing each of the 3 treatment arms to the control group (i.e. without utilizing the factorial design). With the factorial design, if the effects of the two factors are additive, we will be able to detect effect sizes on the order of 25% of one standard deviation. These calculations account for a design effect of 1.25; we expect this to be relatively small, given the small numbers of women sampled per cluster. These effect sizes are small compared to the observed differences between infants reared in more vs. less sanitary environments in observational studies, which were 50-100% of one standard deviation.

The sample size was subsequently increased by allowing all HIV-negative women and their infants who joined the trial from May 1<sup>st</sup> 2014 onwards to enrol, to ensure at least 150 children per trial arm have longitudinal assessments of EED, allowing for missed samples and loss to follow-up. Assuming an average of 2-3 infants per cluster, and a CV of 0.25, this would provide >80% power to detect a shift of 0.25 SD or greater for any individual biomarker between WASH and non-WASH arms, and 0.33 SD between any two trial arms.

### **Identifiers**

As per Main Trial.

## **Risk Factors for Preterm Birth and Small for Gestational Age Sub-study**

### **Study Population**

The study population for this sub-study will consist of pregnancies in women who are enrolled in the trial that are delivered alive either i) at less than 37 completed weeks of gestation (preterm) but appropriate for gestational age (AGA; ie weight >10<sup>th</sup> centile); ii) at term (>37 weeks) but small-for-gestational age (SGA; ie weight <10<sup>th</sup> centile); iii) preterm and SGA or iv) term and AGA (reference group). We will use incidence density sampling to select cases and controls, using the same reference group (term/AGA) for each of the three cases by undertaking group matching, based on maternal HIV status and gestational age at the time of maternal baseline specimen collection.

***Inclusion/Exclusion Criteria***

*Inclusion:* women enrolled in the trial whose infants are SGA/term, SGA/preterm, AGA/preterm (all case groups) compared to women whose infants are AGA/term (control group).

*Exclusion:* women whose infants are AGA/term but not selected as controls.

***Sample Size***

~480-960 women and their infants (240-480 mothers of SGA and preterm infants in the combination groups shown above and a group of control mothers of AGA/term infants). Since we are comparing all case groups with the same control group, we will select a higher number of controls (typically  $\text{SQRT}(k) \times \text{no of cases}$ , where  $k$  = number of case groups). Taking the conservative number of 240, matched 1:1, and assuming a correlation coefficient for exposure between matched cases and controls of 0.2, Types I and II error of 0.05 and 0.80, and a prevalence of 0.25 of a given risk factor among controls, then we will be able to detect an odds ratio of 1.9 or larger (for prevalence of 0.10, detectable OR is 2.4) (Dupont, 1990).

***Identifiers***

As per the Main Trial.

**Risk Factors for Stillbirth Sub-study*****Study Population***

This is a 1:2 case control study that will use incidence density sampling. The study population for this sub-study will consist of outcomes of pregnancies in women who are enrolled in the trial that are delivered stillborn at 28 completed weeks of gestation or later (cases), each matched to 2 pregnancies in women enrolled in the trial that are delivered alive at 28 completed weeks of gestation or later (controls), matched on infant gender, maternal HIV status, and intervention arm. Stillbirth rates are estimated to be 3-4%; thus we will enroll 140-190 cases and 280-380 controls.

***Inclusion/Exclusion Criteria***

*Inclusion:* women enrolled in the trial who deliver a stillborn infant, each matched to 2 control women who did not deliver a stillborn infant.

*Exclusion:* women who deliver at <28 weeks gestation.

***Sample Size***

140-190 stillborn infants and 280-380 of matched controls. Taking the conservative number of 140, matched 1:2, and assuming a correlation coefficient for exposure between matched cases and controls of 0.2, Types I and II error of 0.05 and 0.80, and a prevalence of 0.25 of a given risk factor among controls, then we will be able to detect an odds ratio of 2.3 or larger (for prevalence of 0.10, detectable OR is 3.0) (Dupont, 1990).

***Identifiers***

As per the Main Trial.

## **Risk Factors for Miscarriage Sub-study**

### ***Study Population***

This is a 1:2 case study that will use incidence density sampling. The study population for this sub-study will consist of outcomes of pregnancies in women who are enrolled in the trial and experience spontaneous miscarriage prior to 28 completed weeks of gestation (cases), each matched to 2 pregnancies in women enrolled in the trial who do not experience spontaneous miscarriage prior to 28 completed weeks of gestation (controls), matched on maternal HIV status, and intervention arm. Miscarriage rates are estimated to be 5-10%; thus we will enroll 240-480 cases and 280 – 960 controls.

### ***Inclusion/Exclusion Criteria***

*Inclusion:* women enrolled in the trial who experience spontaneous miscarriage each matched to 2 control women.

*Exclusion:* women who undergo a traditional or medical procedure to induce abortion

### ***Sample Size***

240-480 women experiencing miscarriage and 480 – 960 matched controls. Taking the conservative number of 240, matched 1:2, and assuming a correlation coefficient for exposure between matched cases and controls of 0.2, Types I and II error of 0.05 and 0.80, and a prevalence of 0.25 of a given risk factor among controls, then we will be able to detect an odds ratio of 1.9 or larger (for prevalence of 0.10, detectable OR is 2.4)(Dupont,1990).

### ***Identifiers***

As per the Main Trial.

## **Risk Factors for Neonatal Death Sub-study**

### ***Study Population***

The study population for this sub-study will consist of the mothers of infants who are delivered alive but die within the first 28 days of life from any cause, stratified as early late neonatal deaths, each matched to 2 women enrolled in the trial whose babies are delivered alive and survive the first 28 days of life, matched on infant gender and gestational age, maternal HIV status, and intervention arm. The neonatal death rate is estimated to be around 3%; thus we will enroll approximately 140 cases and approximately 280 controls.

### ***Inclusion/Exclusion Criteria***

*Inclusion:* women enrolled in the trial who deliver a liveborn infant who dies within 28 days of life, each matched to 2 control women on infant gender and gestational age, maternal HIV status, and intervention arm.

*Exclusion:* Infants with life-threatening congenital abnormalities.

### ***Sample Size***

Approximately 140 neonatal deaths and 280 matched controls. Taking the conservative number of 140, matched 1:2, and assuming a correlation coefficient for exposure between matched cases and controls of 0.2, Types I and II error of 0.05 and 0.80, and a prevalence of 0.25 of a given risk factor among controls, then we will be able to detect an odds ratio of 2.3 or larger (for prevalence of 0.10, detectable OR is 3.0) (Dupont, 1990).

**Identifiers**

As per the Main Trial.

## **4. Study Procedures and Design**

### **General Study Design and Methods**

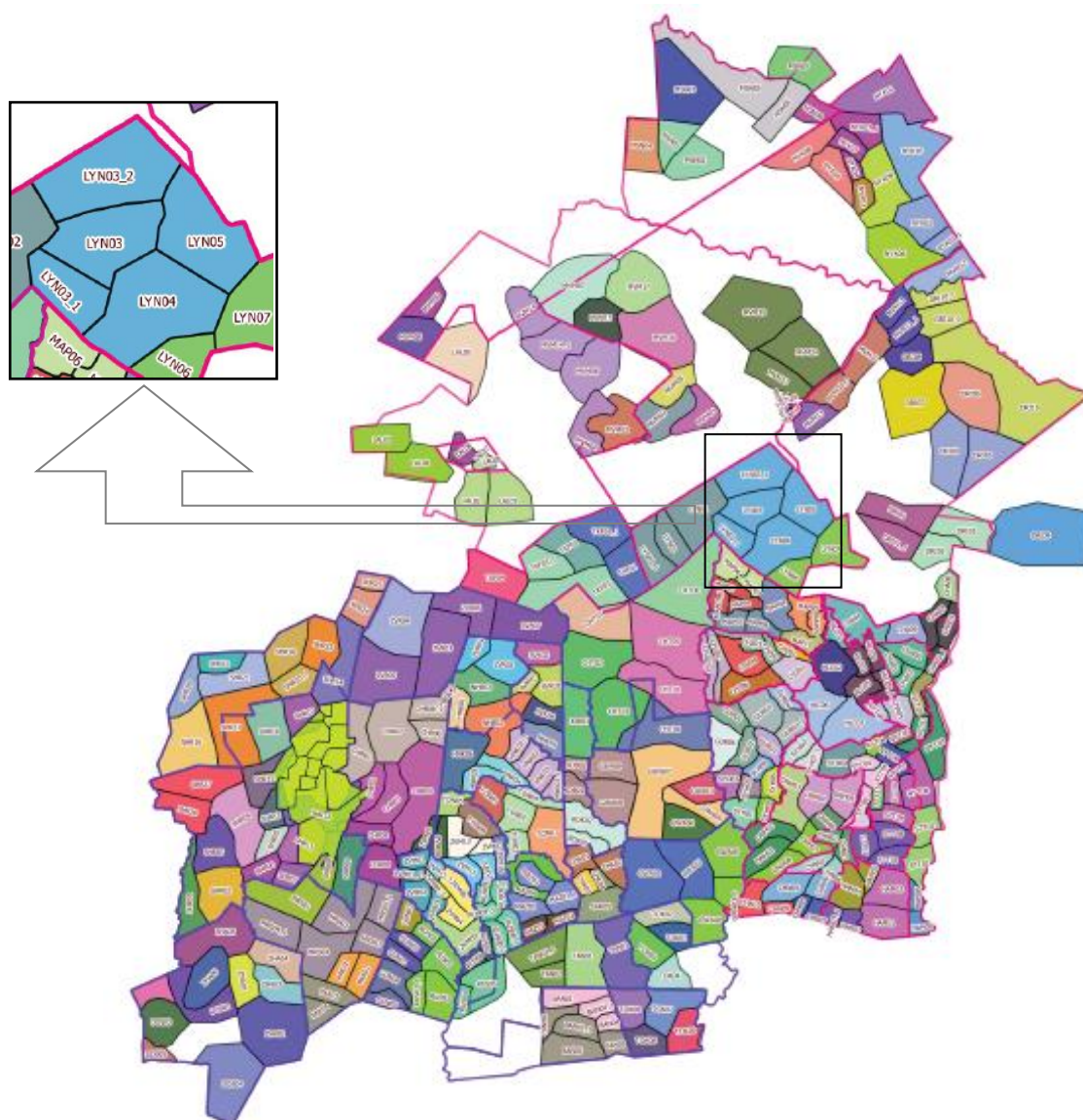
The project will be conducted in two rural districts of Zimbabwe (Chirumanzu and Shurugwi); it will be a community-based, 2X2 factorial cluster-randomized trial among 4800 households in which a woman becomes pregnant during the recruitment period. Our overall approach is to implement a multi-sector project in which randomized interventions are delivered by Village Health Workers who are supervised by Ministry of Health and Child Care (MOHCC) nurses in delivering their regular duties and by research staff in delivering the additional tasks required by the trial. Research staff will measure the quality and extent of delivery of randomized and non-randomized services, behavioral responses to and uptake of the interventions, and trial outcomes. To maintain neutrality of the research staff to the interventions, we will strictly separate the functions and supervision of the Village Health Workers implementing the interventions from the research staff measuring study outcomes.

### **Cluster Construction and Randomization**

#### ***Cluster Definition***

The study area has been divided into 212 clusters, each equivalent to the catchment area of 1 to 3 Village Health Workers. Digital maps of administrative boundaries of the study area were obtained from the Central Statistics Office and using Google Earth, the positions of all homesteads and key landmarks (roads, clinics, schools, rivers) for the 2 districts were plotted. Large scale maps were created including all details and Zvitambo staff met with each of the 320 Village Health Workers and asked them to identify the borders of their catchment areas on the maps. Data were also gathered from the Village Health Worker registers regarding the number of households and reproductive-aged women in their catchment, number of currently pregnant women, and number of <2 year old children. Cluster boundaries were finalized by grouping together Village Health Workers who work together or whose catchment areas include homesteads that are close to the catchment boundaries into the same cluster and putting VHWs that worked alone into one cluster while also attempting to achieve about the same number of households, pregnant women, and young children in each cluster. The resulting map is shown below (Figure 1); where the coloured lines represent ward boundaries and black lines represent VHW boundaries and each different fill color represents a cluster.



**Figure 1 – SHINE clusters****Randomization**

Clusters will be randomized using a highly restricted randomization scheme. Balance will be achieved for key variables to within 5-10 percentage points with balance for each pair of trial arms (6 combinations) and each of the marginal intervention comparison arms (arms 1+2 vs. 3+4 and 1+3 vs. 2+4). The key variables are:

1. Predominant settlement type (traditional communal; resettled)
2. Number of women aged 15-49 years
3. Sanitation coverage (% of households with at least one latrine; % with a latrine that is half or less full; % with a handwashing facility)
4. Water access coverage (% of households with a perennial, functioning water source <500m away; and % with such a source ≥1500m)
5. Mean distance to nearest health facility
6. Geography: balance on mean and variance of latitude and longitude; guarantee each Ward has at least two of the four trial arms represented.

7. District: there will be the same number (within one) of each type of treatment arm in each district (there are 111 units in one district, 101 units in the other, so exact balance within each district is not possible).
8. Village Health Worker characteristics: balance on time-in-post (the VHWs in post received their initial training in either 2001, 2011 or 2012) and number of VHWs in the cluster

Randomization will be conducted by Professor L Moulton using a published method (Moulton, 2004). A computer program will generate millions of random permutations, enumerating those that meet specified balance criteria; a check will be made for bias and validity of the scheme on 5000 such acceptable allocations. Then, from the acceptable allocations that also meet bias and validity specifications, 10 allocations will be randomly selected. Each randomization scheme will divide the 212 randomization units into 4 groups of 53 units. These 10 schemes will be numbered 1-10. Each scheme will be printed on a separate piece of paper with each of the 4 groups of 53 clusters printed in columns labeled A, B, C, and D. The final assignment of the four treatment conditions to the 212 randomization units will be chosen during a forum with community leadership. In their presence, 10 ping pong balls (numbered 1-10) will be placed in an opaque bucket. A representative will select one ball from the bucket held above his/her head. The draw will identify which of the 10 numbered allocations will be used. Then, four balls (labeled A, B, C, and D) will be placed in one bucket, and four balls (labeled with the 4 interventions – see Table below) will be placed in a second bucket. Representatives will draw a ball from the first bucket and a ball from the second bucket, pairing a group of clusters with one of the four intervention arms. This will be repeated twice more to pair the next two groups of clusters with two more intervention arms. The remaining balls in the two buckets will form the final pairing. This second stage is included to provide an additional impression of impartiality/randomness and a further opportunity for participation of the community leadership.

## Intervention Arms

The two active interventions to be studied in this 2X2 Factorial trial are:

- *Intervention 1*: a package of interventions to improve household sanitation and hygiene (WASH)
- *Intervention 2*: a package of interventions to improve infant feeding (IYCF).

The Standard Care interventions are the blanket interventions that were developed and implemented during Phase II of this project and have been approved previously by the IRB. The components of the four intervention arms are detailed in Table 1.

Table 1: Randomized Arms	
<b><u>Standard Care: (Ball labeled “Latrine Later”)</u></b> <ul style="list-style-type: none"> <li>• Exclusive breastfeeding promotion for all infants, birth to 6 months</li> <li>• Strengthened PMTCT services</li> <li>• Strengthened Village Health Worker system</li> <li>• Latrine provided at end of study</li> </ul>	<b><u>WASH: (Ball labeled “Latrine Now”)</u></b> <ul style="list-style-type: none"> <li>• Standard care interventions</li> <li>• Provide household ventilated pit latrine, water treatment solution (Waterguard), monthly liquid soap, play mat and play yard.</li> <li>• Provide interpersonal communication interventions promoting feces disposal in a latrine, HWWS, drinking water treatment, hygienic weaning food preparation, and preventing babies from putting dirt and animal feces in their mouths.</li> </ul>
<b><u>IYCF: (Ball labeled “Latrine Later and Nutributter”)</u></b> <ul style="list-style-type: none"> <li>• Standard care interventions</li> </ul>	<b><u>Sanitation/Hygiene AND Nutrition: (Ball labeled “Latrine Now and Nutributter”)</u></b> <ul style="list-style-type: none"> <li>• Standard care interventions</li> </ul>

<ul style="list-style-type: none"> <li>• Provide 20 g/d Nutributter from 6-18 months</li> <li>• Provide interpersonal communication interventions promoting optimal use of locally available foods for complementary feeding after 6 months, continued breastfeeding and feeding during illness.</li> </ul>	<ul style="list-style-type: none"> <li>• All WASH interventions</li> <li>• All IYCF interventions</li> </ul>
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Interventions will include both commodities (i.e., “hardware”) and behavior change communication (i.e., “software”). These components are described for each type of intervention in Table 2 below:

**Table 2: Components of SHINE interventions**

	IYCF	WASH
Intervention Objective	To achieve dietary intake that meets all nutrient requirements by all children in IYCF clusters everyday between birth and 18 months of life.	To prevent all fecal ingestion by all children in WASH clusters everyday between birth and 18 months of life.
Commodities	Nutributter, 20 g/day from 6-18 months of age	<ul style="list-style-type: none"> <li>• Ventilated pit latrine, or upgrades to existing latrine to meet MOHCC standard</li> <li>• 2 Tippy Tap handwashing facilities</li> <li>• Liquid soap for Tippy Taps</li> <li>• Water Guard water treatment solution</li> <li>• Play mat</li> <li>• Play yard (fence/gate)</li> </ul>
Core Messages	<ol style="list-style-type: none"> <li>1. Feed 20 g Nutributter to your baby every day</li> <li>2. Prepare and feed enriched thick porridge to your baby</li> <li>3. Feed a variety of foods to your baby including animal foods, bean powder, fruits, and vegetables</li> <li>4. Your baby can eat all foods that an adult can eat if it is processed so baby can chew and swallow it</li> <li>5. Continue feeding your baby during and after illness</li> </ol>	<ol style="list-style-type: none"> <li>1. Dispose of all human and animal feces in the latrine</li> <li>2. Wash hands with soap after fecal contact and before preparing, eating or feeding food</li> <li>3. Treat all drinking water with Water Guard</li> <li>4. Put your baby in a clean protected play area where he cannot access dirt and feces when he is playing or eating</li> <li>5. Feed your baby freshly prepared food, or boil leftover food before feeding</li> </ol>

These modules and interactive tools are based on formative research and were piloted during Phase III of this project.

### Measurements and Definitions

A number of parameters will be assessed in this trial that could be measured or defined in a number of ways. Table 3 shows a listing of definitions that will be used in the study. Some of these may be subject to change if more accurate or less costly approaches to measurement become available during the study period.

**Table 3: Definitions used in SHINE**

<b>Parameter</b>	<b>Definition/Assessment Method</b>
Environmental Enteric Dysfunction in Pregnant Women	Assessed through five domains of the hypothesized causal pathway, as outlined on page 5.
Environmental Enteric Dysfunction in Infants	Assessed through five domains of the hypothesized causal pathway, as outlined on page 5.
Gut <u>Inflammation</u> in women and infants	Increased concentrations of fecal neopterin, alpha-1 antitrypsin and myeloperoxidase.
Gut <u>microbial translocation</u> in women and infants	Increased concentrations of plasma Endotoxin Core Antibody, lipopolysaccharide, and soluble CD14
Gut damage/absorptive capacity and permeability in women and infants	Assessed by intestinal fatty acid binding protein (I-FABP), plasma citrulline and lactulose:mannitol ratio
Systemic <u>immune activation</u> in women and infants	Elevated serum levels of CRP, ferritin, CRP, AGP kynurenine:tryptophan ratio and T-cell activation
Infant <u>growth hormone axis</u>	Depressed plasma levels of insulin like growth factor-1 (IGF-1) and collagen X (a marker of growth plate activity)
<u>Aflatoxin exposure</u> in women and infants	Detectable AFB1-lysine in plasma and detectable AFM1 in urine
<u>Fumonisin exposure</u> in women and infants	Detectable FB1 in urine
Deoxynivalenol exposure in women and infants	Detectable DON in urine
Zearalenone exposure in women and infants	Detectable zearalenone in urine
Ochratoxin A exposure in women and infants	Detectable ochratoxin A in urine
T-2 exposure in women and infants	Detectable T-2 in urine
Maternal and infant intestinal microbiota	16S rRNA and whole genome sequencing of DNA and RNA from stool to define the composition and function of the microbial community that inhabits the human intestine.
Maternal anemia	Hemoglobin < 120 g/L as assessed using the Hemocue hemoglobinometer
Maternal severe anemia	Hemoglobin <70 g/L as assessed using the Hemocue hemoglobinometer
Maternal iron status	Plasma ferritin level adjusted for CRP
Infant anemia	Hemoglobin < 105 g/L as assessed using the Hemocue hemoglobinometer
Infant severe anemia	Hemoglobin < 70 g/L as assessed using the Hemocue hemoglobinometer
Infant rotavirus immunogenicity	Measurement of rotavirus IgA titre in plasma
Infant polio vaccine immunogenicity	Measurement of polio virus IgA titre in plasma
Low birth weight	Infant born weighing <2500 g within 72 hours following delivery
Preterm birth	Infant born prior to completing 37 weeks gestation
Stunted for gestational age	Infant with length at birth less than the 10th percentile of WHO standard for term infants (WHO 2006) and a new fetal growth standard which will be released by the end of our trial (Villar 2010)
Small for gestational age	Infant with weight at birth less than the 10th percentile of WHO standard for term infants (WHO 2006) and a new

	fetal growth standard which will be released by the end of our trial (Villar 2010)
IYCF indicators	A set of 7 indicators published by WHO (WHO 2010 )
WASH indicators	Structured observations of key behaviors, and hardware/environment spot-checks to assess WASH behavior uptake: latrine use (presence of fecal matter in the homestead, a well trodden path to the latrine), handwashing (presence of water in the tippy-tap), water treatment (test for residual chlorine in water) and presence of a protected play area
Nutributter	This is a peanut based spread that is fortified with micronutrients and other essential nutrients. See section 11 for complete formulation
Play mat	3 X 2.8 polythene mat
Play yard	A colorful and durable plastic commercially available play yard product. The specific product being distributed is the NorthStates Superyard (Colorplay).
Play space	Play mat + Play yard. The two components are deigned to provide a protective play area for the child that provides protection from environmental contamination while allowing for exploratory play.
Social support for mothering	Assessed using questions adapted from published instruments (Cohen 1985; Sherbourne 1991) and pilot-tested in Shona, comprising material, informational, and emotional support.
Maternal access/control of resources	Questions adapted from Gates-Funded Alive & Thrive Project survey, and pilot-tested in Shona
Maternal depression	Assessed with the Edinburgh Postnatal Depression Scale, whereby 10 questions are each scored between 0-3. A score of 9-11 indicates mild to moderate depression; a score of 12 or above indicates major depression
Maternal stress	Elevated salivary cortisol concentration
Maternal roles, priorities and time	Novel set of questions developed and pilot-tested in Shona by our team
Maternal perceived physical health	Assessed using an adaption of the SF-36
Mothering self-efficacy	Assessed using questions adapted from published instruments (Dumka 1996; Gilmore 2009) and pilot-tested in Shona
Fidelity of implementation (FOI) of interventions	Extent to which study interventions are delivered as designed/intended. (Timely delivery/receipt of intervention messages and inputs; adherence to intervention module structure and content; quality of provider-client interaction/message delivery) Assessed through review of supervision and VHW records to establish timing of VHW visits and adherence to implementation protocols.
Village Health Worker capacity to deliver randomized interventions	VHWs' motivational and supervisory characteristics assessed using questions adapted from IFPRI-World Vision instruments, published instruments and our formative research. Knowledge acquisition and the extent of knowledge sharing with mothers (transfer of knowledge) assessed using knowledge tests administered to mothers and VHWs.

Gross motor, fine motor, language and social development	Assessed using the Malawi Developmental Assessment Tool (MDAT), which measures child development in 4 categories: 1) Motor coordination; 2) Fine motor coordination; 3) Language; 4) Social.
Language development <sup>1</sup>	Assessed using the McArthur Bates CDI: a specific assessment of child language. The interview has three sections: 1) Vocabulary checklist; 2) Communicative gestures; and 3) Grammar checklist.
Object permanence/cognition	Assessed using the A not B test. This task requires the child to watch as a treat is hidden in one of two possible locations; after a brief delay, the infant is allowed to reach for the hidden treat. After two successful retrievals the object is hidden in the alternate location. The exercise is repeated ten times.
Impulsivity	Assessed using the Delayed inhibition test, in which the child is required to watch as a treat is promised to them, but they have to wait to take it.
Maternal-child interaction	Assessed using the Observation of Maternal Child Interaction (OMCI) in which the mother is given a picture book and asked to show her child the book and play with her child normally. The mother is observed and data recorded using a checklist including items on the mother's interaction, mood, verbal statements, language stimulation, the infant's mood, focus, communication and mutual enjoyment.
Home environment and nurturing	Assessed using the Family Care Indicator inventory, which assesses the home environment on child development. The questionnaire/inventory covers: objects children play with in the home; adult-child interactions; the number of books, magazines and newspapers in the home.

<sup>1</sup> The McArthur Bates test is an assessment of language skills, which requires several standardized steps to adapt it for use in a local language. Because there are a very small proportion of Ndebele-speaking families in the study area, the test will be adapted for use in Shona and not Ndebele. Instead, children from Ndebele speaking households will be assessed using the Malawi Developmental Assessment Tool.

### Health System Strengthening Prior to Randomized Trial

The following activities have been conducted under separate phases of this project as preparation for this randomized trial phase. These include:

1. Strengthening of the PMTCT program in the study area.
2. Strengthening of the Village Health Worker program of the Ministry of Health and Child Care.

### *Strengthened Pregnancy Surveillance*

In addition to the above mentioned health systems strengthening activities, prior to beginning trial recruitment, Early Pregnancy Identification (EPregI) by Village Health Workers will be strengthened in the two study districts, in collaboration with the Ministry of Health and Child Care (MOHCC). Currently, MOHCC Village Health Workers are expected to visit every household in their catchment area every 3 months to identify pregnancies, document newly identified pregnancies in the pregnant women's section of the Village Health Worker Register, and refer pregnant women to the nearest health facility for antenatal care by their 4<sup>th</sup> month of gestation (16-24 weeks). Village Health Workers also provide health education to pregnant women and their partners, and discuss the role of men in maternal and newborn health.

However, reliable and timely identification of pregnancies by women and health workers is critical for optimal antenatal care coverage and early pregnancy identification is particularly essential for effective implementation of the 2010 PMTCT Guidance which requires drug initiation by 14 weeks gestation for HIV-positive women. Given the high HIV prevalence in

this setting, the MOHCC has agreed to work in collaboration with our team to strengthen pregnancy surveillance by systematizing the record keeping by VHWs and by training them, as a routine part of their work, to enquire about a woman's last menstrual period (LMP) and to offer urine dipstick pregnancy tests to mothers in the community who have missed a period. To assist women in recalling their LMP date, VHWs will provide all reproductive-aged women with a calendar, which can be used to record her menstruation dates. In addition, women will be given a generic information sheet which provides an overview of the SHINE trial. Women who have a positive pregnancy test will be given a referral slip to the health center, encouraged to book as soon as possible, and encouraged to be HIV-tested at the first ANC visit. For women who have a negative pregnancy test followed by a positive pregnancy test, or who can provide a reliable LMP date, the VHW will also request the woman's assent be visited by a SHINE Data Collector (DC) to learn about SHINE, following the script on the Village Health Worker Weekly Form (**SHINE\_Form1\_v3\_VHW Weekly Form and Script to Assent for Referral to SHINE**). If the mother assents, the Village Health Worker will tell the mother that he/she will return within a few weeks with a SHINE Data Collector. The SHINE field hub will receive an SMS from the VHW once s/he has identified a newly pregnant woman. S/he will include in her SMS, following the woman's verbal assent, the date of her LMP so that gestational age can be calculated. Women identified as pregnant will be eligible to join SHINE. The SHINE Field Data Supervisor will schedule an appointment date for the VHW and a SHINE Data Collector (DC) to go together to the woman's house. The VHW will leave **an Intervention-specific brochure (SHINE\_16\_v2 WASH Brochure; SHINE\_17\_v2 WASH/IYCF Brochure; SHINE\_18\_v2 IYCF Brochure; SHINE\_19\_v2 SOC Brochure)** with the woman, which provides further information about the SHINE intervention the mother would receive if she joined, based on the cluster where she lives. At the screening visit, the DC will explain the SHINE trial to the mother if she is eligible (ie confirmed pregnant by a urine pregnancy test)

### Antenatal Clinic Screening

We will also screen women attending antenatal clinics to identify eligible women who may have been missed by E Preg I. A SHINE data collector will visit high volume ANC sites. After introduction by the Sister in Charge, the DC will ask the women attending for antenatal care whether they know about SHINE, provide them with the community brochure describing the trial, and complete the SHINE-Form 27\_v1 Surveillance Form to identify eligible women who are interested in joining SHINE. For women who are interested in the trial, the DC will make an appointment with her for a consent visit at her home by calling the Field Data Supervisor at the SHINE hub.

The Field Data Supervisor will also contact the woman's VHW and request the VHW to meet the Data Collector for the consent visit. Thereafter, consent into SHINE will proceed in the usual way as described above.

### Screening and referral for postnatal treatment of schistosomiasis

In mid-August 2011, the findings of a national helminthiasis and schistosomiasis survey, which had been conducted at the district level in late 2010 by the Zimbabwean National Institutes of Health Research, were released. (Ministry of Health and Child Care of Zimbabwe, 2011). In brief, 10,222 school-age children were tested for soil transmitted helminths (hookworm+ ascaris+ trichiuris) and schistosomiasis [*S. hematobium* (urinary) and *S. mansoni* (intestinal)]. Prevalence for our study districts are presented in Table 4.

**Table 4: Prevalence of schistosomiasis and soil-transmitted helminths in school-age children in Shurugwi and Chirumanzu.**

Study district	Schistosomiasis	Soil transmitted helminths
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Shurugwi	55.9%	1.5%
Chirumanzu	23.5%	0%

Shurugwi had one of the highest prevalence of schistosomiasis in the country; nearly all of the infections were urinary (only 0.8% intestinal). Based on these survey findings, the WHO recommendations are annual and biannual mass drug administration for Shurugwi and Chirumanzu, respectively. The first mass drug administration is due to be delivered by MOHCC in June 2012, which will coincide with the start of SHINE. All members of the community will be treated presumptively, except for women who are identified as being pregnant based on a history of amenorrhea. Although a WHO informal consultation (WHO, 2002) recommended that pregnant and lactating women should benefit from treatment with praziquantel as much as any other group, this guidance has not been adopted by MOHCC in Zimbabwe because of concerns regarding abortion following administration of praziquantel.

Reproductive aged women have been identified as a high-risk group by WHO, because of the morbidity caused by female genital schistosomiasis and anemia, together with the potential for adverse birth outcomes among infected women.

While schistosomiasis prevalence is likely to be lower in adults than primary school children, these survey findings suggest schistosomiasis may be both substantial and an important cause of anemia and immune activation in pregnant women enrolled in our trial. Since both maternal anemia and immune activation are likely to mediate adverse birth outcomes, maternal schistosomiasis is a potentially important factor in this current proposal. The majority of women recruited to the trial will have received praziquantel during the mass drug administration, therefore we anticipate the prevalence of schistosomiasis will be substantially reduced. However, treatment failure and re-infection occurs commonly in endemic areas. We will therefore screen all women recruited to the trial for schistosomiasis by urinary filtration and light microscopy for egg detection, to determine the prevalence of the infection among women and to enable us to adjust for schistosomiasis infection in our analyses. Any women found to be infected will be informed and advised to seek treatment from their local clinic after delivery.

### **Screening for mycotoxin exposure among antenatal women**

Mycotoxins are fungal metabolites that can contaminate foods such as maize, wheat and groundnuts. Three mycotoxins that are particularly prevalent in food, and which have been linked to poor growth in humans and/or animals, are aflatoxin, fumonisin and deoxynivalenol (DON). Additionally, recent method development allows the assessment of other potentially important mycotoxins in urine including: *T-2-toxin (T-2) (and its metabolites HT-2-toxin (HT-2), HT-2-toxin-4-glucuronide (HT-2-4-GlcA)), zearalenone (ZEA) (and its metabolites zearalanone (ZAN),  $\alpha$ -zearalanol ( $\alpha$ -ZEL), and  $\beta$ -zearalanol ( $\beta$ -ZEL) and corresponding 14-O-glucuronic acid conjugates (ZEA-14-GlcA, ZAN-14-GlcA,  $\alpha/\beta$ -ZEL-14-GlcA)) and ochratoxin A (OTA and ochratoxin alpha (OT $\alpha$ )).* One of the pathological mechanisms underlying mycotoxin exposure is intestinal damage, which is histologically similar to EED. Several studies have shown associations between maternal aflatoxin exposure and low birth weight, and between infant aflatoxin exposure and stunting in early childhood. Little is known about the impact of fumonisin or DON on adverse birth outcomes or stunting, but it is biologically plausible that these mycotoxins may mediate similar effects to aflatoxin. Assessment of T-2 toxin, zearalenone, and ochratoxin A will allow us to estimate the total mycotoxin exposure burden in SHINE participants. The prevalence of mycotoxin exposure in Shurugwi and Chirumanzu is not known, but since the staple foods in these districts include maize and ground nuts, there may be high levels of exposure among antenatal women and their infants. To determine the levels of exposure, all 4800 mothers will be screened for



aflatoxin M1 at the baseline and 30-34 weeks gestation visits, and the 1600 infants enrolled in the EED substudy will be assessed for aflatoxin M1 at 3, 6, 12 and 18 mo and aflatoxin-lysine fumonisin, DON, T-2 toxin, zearalenone and ochratoxin A at 6, 12 and 18 mo of age. Mothers in the preterm birth/SGA substudy will be assessed for aflatoxin-lysine fumonisin, DON, T-2 toxin, zearalenone and ochratoxin A

Methods development for the aflatoxin-albumin ELISA and mass spectrometry to measure exposure in SHINE trial participants in Zimbabwe have been ongoing, but there is a global lack of standard materials (i.e. samples with known concentrations of aflatoxin-albumin). After 2 years of working on these methods, we have failed to develop a sufficiently sensitive analytical method with the equipment available in Zimbabwe and must ship samples for analysis to University of Maryland College Park, USA and/or University of Muenster, which have the technical mass spectrometry capacity to measure aflatoxin-lysine. Aflatoxin-lysine will be extracted from maternal and infant plasma samples using solid-phase extraction columns in the Zvitambo laboratory, and the extracted aflatoxin-lysine analyte will be shipped to University of Maryland College Park, , USA and/or University of Muenster, Germany to measure aflatoxin-lysine using mass spectrometry.

The equipment in Zimbabwe will subsequently not be suitable for measurement of urinary excretion of mycotoxins and their metabolites. Recently, a lab at the University of Muenster has developed a highly sensitive method to measure multiple mycotoxin exposure. Urine samples will be shipped to the University of Muenster, Germany to measure fumonisin, DON, T-2 toxin, zearalenone and ochratoxin A.

## **Study Procedures in Sequential Order for Women Referred to Study**

### ***Informed Consent Visit***

The DC will ask the mother whether she has received an **intervention-arm specific brochure (ie, SHINE forms 16, 17, 18, or 19)** and give her one if she does not have it. The DC will review the brochure with the mother, allowing her to ask questions.

Note that due to the clustered nature of the randomization and that many of the interventions will be delivered through the Village Health Worker System, the consent form will be customized for women in each intervention cluster [(See **Consent Forms specific to each intervention arm: SHINE\_Form 2\_v10\_SOC Consent Form; SHINE\_Form 3\_v10\_IYCF Consent Form; SHINE\_Form 4\_v10\_WASH Consent Form; SHINE\_Form 5\_v10\_WASH+IYCF Consent Form**].

The DC will tell the woman that only women who are pregnant are eligible for enrollment into SHINE. Following the intervention-specific consent form, the DC will obtain the mother's written permission to transcribe the date of her LMP from the Village Health Worker's register, and to confirm her pregnancy with a second urine pregnancy test. The DC will also enquire about any episodes of vaginal bleeding since the VHW undertook a pregnancy test. If the pregnancy test is negative the nurse will provide counseling regarding causes of early pregnancy loss or amenorrhea not associated with pregnancy, and tell the mother that if she becomes pregnant again during the recruitment period of the trial, she would then be eligible for the trial. If the woman reports a recent episode of vaginal bleeding, the nurse will explain that this could be a sign of early pregnancy loss and that the pregnancy test can remain positive for several weeks after a miscarriage. The DC will not enroll the woman at that visit, but will refer her to the local clinic for assessment. She will reschedule an appointment 2 weeks later, when she will enquire about the medical assessment of the mother's bleeding. If early pregnancy loss was confirmed by the clinic, she will be counseled but not enrolled into the trial; the DC will tell the mother that if she becomes pregnant again during the recruitment period of the trial, she would then be eligible for the trial. If early pregnancy loss was not confirmed by the clinic, she will be offered another pregnancy test. If the test is

positive and she has had no further episodes of vaginal bleeding since her clinic appointment, and she is <24 gestational weeks by calculation from the LMP, the DC will describe the study following the intervention-specific consent form. If the mother wants more time to decide (i.e., wishes to discuss the study with other family members or consider it further) the DC will leave the intervention-specific consent form with the mother and make an appointment for a return visit. If the mother does not consent, the DC requests the mother's assent to record her age, place of residence (Study cluster number), and reason for not joining the study on a Declining Participant Information Sheet (**SHINE\_Form 6\_v4\_Declining Participant Information Sheet**)

If the mother consents to join the study, the DC will proceed to obtain consent for specimen archival, using the (**SHINE\_Form 9\_v5\_Specimen Storage and Shipment Consent Form**). The DC will then schedule a baseline visit with the mother and give her a labeled stool collection package and ask her to collect a sample of her stool on the morning of the baseline visit if possible.

At each postnatal research visit a small, non-monetary gift will be offered to the mother to thank her and her infant for their time in participating in data collection. The total value of all gifts over the course of the study will not exceed \$10.

### ***Baseline Enrollment Visit***

On the appointed day, the DC will arrive at the mother's house in the morning and proceed as follows:

- If the mother has produced a stool sample that morning, the DC collects it and stores it in a cool box. If she has not provided a stool sample, the nurse will ask her to try to provide a stool sample before ingesting the lactulose-mannitol solution. If she is not able, the DC leaves the specimen container with the mother and will schedule a time to come and collect the sample, if feasible.
- The DC asks the mother to provide a urine specimen before ingestion of lactulose-mannitol solution, and undertakes urinalysis using a Multistix test strip. She also stores a sample of this urine in the cool box for subsequent microscopy and storage.
- The DC asks the mother to drink a solution of Lactulose and Mannitol (sugar water) and records the time of consumption on the Baseline Survey Form (**SHINE\_13\_v5\_Baseline Survey Form**).
- For 2 hours, the nurse collects and measures all maternal urine, storing the specimen in a pre-labeled bottle in the cool box.
- During the 2-hour urine collection period, the nurse:
  - Administers the Baseline Survey Form; see Research Data Collection section for details).
  - Weighs the mother and measures her height and mid-upper arm circumference (MUAC)
  - Weighs the mother's youngest child <24 months of age and measures his/her recumbent length and MUAC
  - Addresses any questions about the study which the mother or a member of her household has
  - Collects maternal blood samples by venous puncture (5 ml in clot tube, 2 x 5 ml in EDTA tubes)
  - Tests the mother's blood for HIV using a rapid test algorithm approved for use in Zimbabwe and provides appropriate counseling (referral for PMTCT care if positive, how to stay negative if negative). If the mother prefers not to have the HIV test in her home, the DC discusses the benefits of learning her HIV status during early pregnancy so that if she is positive she can receive medication to help prevent her

baby from getting infected, and will strongly encourage her to be tested during her first visit to antenatal clinic. In all Zimbabwean public antenatal clinics, HIV testing is done routinely for all pregnant women at first booking (unless the woman specifically “opts out”) and HIV test results are recorded on the woman’s handheld antenatal card. For women who opt out of HIV testing, the DC will explain that HIV testing will be undertaken on her blood sample in the Zvitambo laboratory, because it is important for the trial to know her HIV status; however, she can choose not to receive the result if she prefers.

- Tests the mother’s blood for hemoglobin using the Hemacue rapid test. If the mother’s hemoglobin is <10g/dL the DC will refer the mother for work-up and care at the local clinic. Regardless of the hemoglobin result, the DC will explain the advantages of iron and folic acid (IFA) supplementation during pregnancy and advise early booking for antenatal care to access IFA.
- Measures the mother’s blood pressure
- Collects saliva sample
- Makes an appointment with the mother for the 32 weeks gestation follow up visit. The target timing of the 32 week gestation visit is 30-34 weeks; if the baseline visit occurs >30 weeks, the 32 week gestation visit will be omitted.

### ***Intervention and Research Data Collection Activities Timeline***

Following the baseline visit, two sets of activities will be initiated; 1) the interventions based on the randomization and 2) continued research data collection. The staff who implement the interventions are purposely separate from the staff who will conduct research data collection. All the education and behavior change components of the intervention modules will be implemented through the MOHCC Village Health Worker system with the support of SHINE Intervention Nurses (INs). The construction of latrines and installation of hand washing stations (Tippy Taps) in the WASH arms will be done through a collaboration between Zvitambo and the Ministry of Health and Child Care.

The table below illustrates how these two sets of activities are integrated temporally. In the following sections we specify the components of each of the behavior change modules referred to in Table 5 (all of which were developed and pilot tested during previous phases of this project following IRB approval) and the content of each data collection visit.

**Table 5 – Timetable of research and implementation visits in SHINE**

RESEARCH		IMPLEMENTATION				
Target date (optimal dates) [allowable dates]§	Data collection activity	Timing of intervention visit Intervention Implementation Activities Black – MOHCC intervention delivered by Village Health Worker Blue – Study behavior change intervention delivered by Village Health Worker Red – Study hardware intervention delivered by MOHCC/study staff Green – Study consumable delivery by Village Health Worker				
		Target date* (optimal dates) [allowable dates]	Standard Care	IYCF	WASH	IYCF+WASH
2-<24 weeks gestation	Village Health Worker referral to trial		Village Health Worker referral for ANC booking	Village Health Worker referral for ANC booking	Village Health Worker referral for ANC booking	Village Health Worker referral for ANC booking
9 weeks (8-12 weeks) [8-wks gestation < parturition]	Informed consent and enrolment					
11 weeks (10-12 weeks) [10wks gestation<part urition]	Baseline visit <b>SHINE_Form 13_ v5 _Baseline form</b>					

		≤24 wks gestation (before delivery)			<ul style="list-style-type: none"> <li>• Latrine construction</li> <li>• 2 Tippy Taps delivered to household</li> </ul>	<ul style="list-style-type: none"> <li>• Latrine construction</li> <li>• 2 Tippy Taps delivered to household</li> </ul>
		25 wks gestation (25-<29 wks) [Before delivery]	• Module S1	• Module S1	<ul style="list-style-type: none"> <li>• Module S1</li> <li>• Module W1</li> </ul>	<ul style="list-style-type: none"> <li>• Module S1</li> <li>• Module W1</li> </ul>
		29 wks gestation (29-<33 wks)	• Module S1:	• Module S1	<ul style="list-style-type: none"> <li>• Module W2</li> <li>• Give soap.</li> <li>• Module S1</li> </ul>	<ul style="list-style-type: none"> <li>• Module W2</li> <li>• Give soap.</li> <li>• Module S1</li> </ul>
32 wks gestation (30-34 weeks) [30 weeks gestation<parturition]	Pregnancy Follow-up Visit <b>SHINE_Form 15_v2_32 weeks Gestation form</b>	33 wks gestation (33-37 wks) [Before delivery]	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S1</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S1</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Give soap</li> <li>• Module S1</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Give soap</li> <li>• Module S1</li> </ul>
BIRTH (0-1 week) [0-4 weeks]	Birth visit** <b>SHINE Form 25_v1_Birth Visit_</b>	BIRTH Day 3	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S2</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S2</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S2</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S2</li> </ul>
5 weeks (4-6 weeks) [4-12 weeks]	End of Pregnancy <b>SHINE_Form 14_v5 End of Pregnancy form / 1 month visit***</b>	3 weeks (3-<8 weeks) /1 month visit	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> <li>• Give soap</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> <li>• Give soap</li> </ul>
		8 weeks (8-<12 weeks) /2 month visit	• Module S3	• Module S3	<ul style="list-style-type: none"> <li>• Module W3</li> <li>• Module S3</li> <li>• Give soap</li> </ul>	<ul style="list-style-type: none"> <li>• Module W3</li> <li>• Module S3</li> <li>• Give soap</li> </ul>
14 weeks (12-16 weeks)	3 month visit	12 weeks (12-<16 weeks)	• EBF Module	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> </ul>	<ul style="list-style-type: none"> <li>• EBF Module</li> <li>• Module S3</li> </ul>

[12-25 weeks]	<b>SHINE_Form 20_v4_3mo visit</b>	/3 month visit	• <b>Module S3</b>		• Give soap	• Give soap
		16 weeks (16-<20 weeks) /4 month visit	• <b>Module S4</b>	• <b>Module S4</b>	• <b>Module W4</b> • Give Water Guard and soap • <b>Module S4</b>	• <b>Module W4</b> • Give Water Guard and soap • <b>Module S4</b>
		21 weeks (21-<25 weeks) /5 month visit	• <b>Module S4</b>	• <b>Module N1</b> • <b>Module S4</b>	• <b>Module W5</b> • Give Water Guard and soap • <b>Module S4</b>	• <b>Module N1</b> • <b>Module W5</b> • Give Water Guard and soap • <b>Module S4</b>
		21 weeks (21-<33 weeks)			• Play yard delivered to household	• Play yard delivered to household
27 weeks (25-29 weeks) [25-51 weeks]	6 month visit <b>SHINE_Form 21_v3_6mo visit</b>	25 weeks (25-<29 weeks) /6 month visit	• <b>Module S4</b>	• <b>Module N2</b> • Give Nutributter • <b>Module S4</b>	• Give Water Guard and soap • <b>Module S4</b>	• <b>Module N2. Infant's first solid foods</b> • Give Nutributter • Give Water Guard and soap • <b>Module S4</b>
		29 weeks (29-33 weeks) /7 month visit	• <b>Module S4</b>	• <b>Module N3</b> • <b>Module S4</b> • Give Nutributter •	• <b>Module S4</b> • Give Water Guard and soap •	• <b>Module N3</b> • <b>Module S4</b> • Give Nutributter • Give Water Guard and soap •
		34 weeks (34-<38 weeks) /8 month visit	• <b>Module S3</b>	• <b>Module N4</b> • <b>Module S3</b> • Give Nutributter •	• <b>Module S3</b> • Give Water Guard and soap •	• <b>Module N4</b> • <b>Module S3</b> • Give Nutributter • Give Water Guard and soap •

		42 weeks (42-46 weeks) /10 month visit	• <b>Module S3</b>	<ul style="list-style-type: none"> <li>• <b>Module N5</b></li> <li>• <b>Module S3</b></li> <li>• Give Nutributter</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Module S3</b></li> <li>• Give Water Guard and soap</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Module N5</b></li> <li>• <b>Module S3</b></li> <li>• Give Nutributter</li> <li>• Give Water Guard and soap</li> <li>•</li> </ul>
		47 weeks (47-<51 weeks) /11 month visit	• <b>Module S4</b>	<ul style="list-style-type: none"> <li>• <b>Module S4</b></li> <li>• Give Nutributter</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Module W4</b></li> <li>• <b>Module S4</b></li> <li>• Give Water Guard and soap</li> <li>•</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Module W4</b></li> <li>• <b>Module S4</b></li> <li>• Give Nutributter</li> <li>• Give Water Guard and soap</li> <li>•</li> </ul>
53 weeks (51-55 weeks) [51-76 weeks]	12 month visit <b>SHINE_Form 22_v2_12mo visit</b>	51 weeks (51-55 weeks) /12 month visit	Review S4	Review Review S4 • Give Nutributter	Review Review S4 • Give Water Guard and soap	Review Review S4 • Give Nutributter • Give Water Guard and soap
		13-17 monthly visits		• Give Nutributter	• Give Water Guard and soap	• Give Nutributter • Give Water Guard and soap
78 weeks (76-80 weeks) [76-130 weeks]	18 month visit <b>SHINE_Form 23_v2_18mo visit</b>	77 weeks (77-<81 weeks) /18 month visit	• <b>Module S3</b>	• <b>Module S3</b>	• <b>Module S3</b>	<ul style="list-style-type: none"> <li>• <b>Module S3</b></li> <li>•</li> </ul>
104 weeks (102-106 weeks) [102-110 weeks]	24 month visit <b>SHINE_Form 63 v5_24 month visit form.</b>					



\*Date stated is the target date. VHW visits should ideally be made within a week of the target date, but can be made within a 4-week window of this date, as shown in brackets. Missed VHW module delivery visits may be summarized during subsequent contacts. Timing of VHW visits will be monitored to establish the degree of intervention fidelity. The target for latrine construction and tippy tap distribution is <24 weeks gestation, but in all cases the WASH hardware must be in place before the baby is born. The target for play yard distribution is 21 weeks of child's age, but in all cases the play yard must be at the household before the child is 33 weeks of age.

\*\*Birth visit is for home deliveries only, to undertake anthropometry on the infant using SHINE\_Form 25\_v1\_Birth Visit. In the case of institutional deliveries, anthropometry will be undertaken in the health facility, and the birth home visit will not be made.

\*\*\*The 1 month postnatal visit will usually coincide with the end of pregnancy visit. In cases of miscarriage, premature delivery, stillbirth, neonatal death or maternal death, an end of pregnancy visit will be scheduled as soon as feasible after the Hub is informed, and in the event the baby was delivered at an institution, further birth information will be collected using SHINE\_Form 55\_v2\_Birth Facility Information.

§ If the participant is away for a long period of time and is not able to be visited within the allowable window, then a decision may be made by the Hub to obtain data for that visit at a subsequent time-point to avoid missing data, using SHINE\_Form 54v1 End of Pregnancy Catch Up Form and SHINE\_Form 56v1\_Baseline Catch up Form for EOP and baseline data respectively.

**Behavior Change Intervention Module Description**

All behavior change interventions will be delivered by the Village Health Worker. The components of each module are identified in Table 6.

**Table 6 – Behavior change intervention modules**

<b>Module Number</b>	<b>Components</b>
<b>W1</b>	<b>Stool Disposal</b> <ul style="list-style-type: none"> <li>Emphasizes extent of fecal contamination (human and animal) in the household</li> <li>Discuss the importance of maintaining a clean environment for baby's health</li> <li>Promoting consistent latrine use by all household members</li> <li>Discuss importance of proper stool disposal in relation to baby's health</li> </ul>
<b>W2</b>	<b>Handwashing with soap (HWWS)</b> <ul style="list-style-type: none"> <li>Initiate hand washing with soap discussion <ul style="list-style-type: none"> <li>HWWS after handling fecal material</li> <li>HWWS before handling food</li> <li>HWWS of the infant's hands</li> </ul> </li> <li>Emphasis on disrupting the transmission of germs to the infant</li> <li>Discuss how to use the tippy tap</li> </ul>
<b>W3</b>	<b>Protection of the Developing Child</b> <ul style="list-style-type: none"> <li>Discuss what the 'traffic of dirt' mean in terms of the amount of fecal material in the soil</li> <li>Using a Play mat and play yard to prevent children from eating soil or chicken feces</li> </ul>
<b>W4</b>	<b>Water treatment</b> <ul style="list-style-type: none"> <li>Discussion of the importance of water treatment</li> <li>Discuss water treatment</li> </ul>
<b>W5</b>	<b>Hygienic Handling Preparation and Feeding of Complementary Food</b> <ul style="list-style-type: none"> <li>Discuss hygienic preparation of infant food, <ul style="list-style-type: none"> <li>clean utensils,</li> <li>HWWS before serving food</li> <li>hygienic storage</li> <li>reheating of food that was safely stored</li> </ul> </li> </ul>
<b>EBF</b>	<b>Exclusive breastfeeding</b> <ul style="list-style-type: none"> <li>All infants should be fed only breastmilk from birth to 6 months</li> <li>This means no water, no cooking oil, no traditional medicines – nothing but breastmilk (emphasis on the management of fontanelle, colic)</li> <li>EBF is important for all infants whether mother is HIV positive or HIV negative.</li> </ul>
<b>N1</b>	<b>Nutrition for your baby</b> <ul style="list-style-type: none"> <li>Emphasizes the importance of continuing to exclusively breastfeed infants from 5 to 6 months of age</li> <li>Discuss the various roles that food helps the body perform: brain development, fight infection, health growth</li> <li>Responsive Feeding</li> </ul>
<b>N2</b>	<b>Your baby's first solid foods</b> <ul style="list-style-type: none"> <li>Introduction of solid foods: Nutributter and thick porridge</li> <li>Why does the infant now need foods in addition to breast milk</li> <li>How much food can be fed to the infant</li> <li>Demonstrate how to prepare thick porridge</li> <li>Demonstrate how to feed infant</li> </ul>

<b>N3</b>	<b>Introducing more foods to your baby</b> <ul style="list-style-type: none"> <li>• Emphasize breast milk as the foundation of infant's diet</li> <li>• Importance of continuing to feed 1 sachet of Nutributter each day</li> <li>• Importance of adding a variety of other foods to the infant's diet</li> <li>• Demonstration of food processing</li> <li>• Review and support of responsive feeding</li> </ul>
<b>N4</b>	<b>Maintenance of breastfeeding and feeding during illness</b> <ul style="list-style-type: none"> <li>• Discussion of what mother feeds infant during illness</li> <li>• Explanation of what infant needs during illness to recover</li> <li>• How to feed infant during illness</li> <li>• How to address diarrhea</li> </ul>
<b>N5</b>	<b>Dietary Diversity</b> <ul style="list-style-type: none"> <li>• Importance of varying infant's diet now that it is older</li> <li>• Discussion of 4 main food groups: porridge/sadza (carbohydrates), meat (protein), vegetable (vitamins/minerals), cooking oil/sugar (extra energy)</li> <li>• Discussion of foods mother prepares for the family</li> <li>• Review of responsive feeding</li> </ul>
<b>S1</b>	<b>Encouraging Targeted ANC visit</b> <ul style="list-style-type: none"> <li>• Introduction to the antenatal care clinic</li> <li>• Discussion of what to expect at a visit</li> <li>• Importance of Iron Folate supplements</li> <li>• Importance of Tetanus Injection</li> </ul>
<b>S2</b>	<b>Early Neonatal Home Visit</b> <ul style="list-style-type: none"> <li>• Introduces and promotes skin to skin contact</li> <li>• Promotes hygienic umbilical cord care</li> <li>• Provides support for low birth weight and sick newborns and infants born to HIV infected mothers</li> </ul>
<b>S3</b>	<b>EPI Reminders</b> <ul style="list-style-type: none"> <li>• Emphasizes the importance of vaccinations</li> <li>• Encourages timely vaccinations according to the national EPI schedule</li> </ul>
<b>S4</b>	<b>Family Planning</b> <ul style="list-style-type: none"> <li>• Introduces Family Planning</li> <li>• Timed discussion of various Family Planning methods</li> </ul>

### Qualitative study of mothers' experiences in receiving the SHINE interventions.

At least one year after the recruitment of mothers, we will purposively sample up to 80 mothers (20/arm) who have been enrolled in SHINE, to conduct an in-depth interview. The purpose of the interview is to learn about what mothers think about the interventions in SHINE and the performance of VHWs i.e. the different ways the VHWs deliver intervention messages.

This exercise may lead to improvements in the interventions or their delivery. A SHINE researcher will visit the mother and obtain her consent for the interview using SHINE Form 33 v1 Mother's Intervention Delivery Perception Consent Form. The interview (using SHINE Form 34v1 Mother's Intervention Delivery Perception Questionnaire) will last up to 2 hours and will be audiotape recorded if the mother provides permission. If information has been missed in the interview, the mother may have one follow-up to gather that information missed in the initial interview. All audiotapes will be destroyed after transcription.

### **Qualitative study to assess the potential effectiveness of the SHINE WASH intervention**

It is important to understand not only the positive and intentional impact of the intervention but also to understand what unintended consequences might result. To elucidate this, and in order to strengthen the delivery of the module on "Protection of the Developing Child" (W3), we will conduct structured interviews and observations among a purposive sample of up to 40 women enrolled into the WASH and WASH + IYCF arms and 40 women enrolled in the IYCF and SOC arms of the study and with a baby between 5-18 months. Structured interviews and observations will enable us to:

- Confirm the feasibility and acceptability of the play space in this context;
- Learn how play spaces are being used and where, the frequency and duration of their use, and their cleaning and storage;
- Assess if the intervention is introducing unintended benefits or risks;
- Ascertain and address any safety concerns or misunderstandings;
- Assess if the interventions modify the care that babies receive; and
- Assess if mother's perspectives about child care are influenced by the interventions they are receiving

Two rounds of data collection visits will be conducted: the first round will target women with babies 5-8 months and who would have recently received play yards, while the second round will target women with babies 12-18 months (at least 6 months after the delivery of the play space).

The first round of data collection will comprise two visits conducted among up to 40 women.

**Visit 1:** 1-3 weeks after the delivery of the play yard SHINE research staff will obtain written informed consent using **SHINE Form 36v1 developmental evaluation consent form**. The researcher will ask the mother questions related to, and make observations around the use of the SHINE play space components (play mat and play yard) using **SHINE Form 37 v1 play space developmental evaluation Visit 1**. Based on their observations, the researcher will also counsel the mother on safe use of the play space and potential problems and suggested solutions using SHINE form 38 v1 play space counselling guide. The visit will last up to 2 hours and the participant interviews will be audio/tape recorded if the mother provides permission. All audiotapes will be destroyed after transcription.

**Visit 2:** 2 weeks after visit 1, SHINE research staff will conduct a follow-up visit to ask further questions and make observations around the continued use of the play space using **SHINE form 39 v1 play space developmental evaluation Visit 2**. The visit will last up to 2 hours and the participant interviews will be audio/tape recorded if the mother provides permission.

For the second round of data collection (to be done at least 6 months after the first round) we will select another 80 women (who may include round one participants) and visit them once. During this visit, SHINE research staff will obtain written informed consent using SHINE Form 36 v1 developmental evaluation consent form (for new, non-round-one, participants) and collect questionnaire and observation data using **SHINE form 37 v1 play space developmental evaluation Visit 1**. The observation will last up to 6 hours and the

interview up to an hour. The interview will be audio/tape recorded if the mother provides permission.

### **Qualitative Study of Intervention Nurse evaluation of VHW performance**

In order to strengthen the ongoing supportive supervision of VHWs, we will conduct in-depth interviews among a purposive sample of up to 10 intervention nurses. The interviews will provide insight into the intervention nurses' (IN) perspective of VHW performance and identify target areas to more effectively support VHWs in their SHINE activities. A SHINE researcher will visit the IN and obtain consent using **SHINE form 52v1 Intervention Nurse's perception of VHW performance**. The interview will last up to 1 hour. All interviews will be audiotape recorded if the IN provides permission. All audiotapes will be destroyed after transcription.

Qualitative study of mothers' experiences using the SHINE

interventions.

We will purposively sample up to 80 mothers (20/arm) who have been enrolled in SHINE, to conduct an in-depth interview.

The purpose of the interview is to learn about mother's' experiences using the SHINE interventions and get their insights on challenges and opportunities for using these interventions. This exercise will explore how the SHINE interventions fit into the existing child care environment and how they influenced the mother's child care decisions. A SHINE researcher will visit the mother and obtain her consent for the interview using SHINE\_ Form 76\_ v1\_ Mother's Experience with Interventions Consent Form. Following consent the researcher will conduct an in-depth interview using SHINE\_ Form 77\_v1\_ Mother's Experience with SHINE Interventions Questionnaire. The interview will last up to 2 hours and will be audiotape recorded if the mother provides permission. If information has been missed in the interview, the mother may have one follow-up to gather that information missed in the initial interview. All audiotapes will be destroyed after transcription.

### ***Research Data Collection***

All data collection is conducted by DCs during household visits, except institutional birth data which is transcribed from health registers: a) for deliveries that occur in one of the 43 health institutions in the study area, birth data will be transcribed by the DC from the Ministry of Health and Child Care delivery register; b) for home deliveries, birth data will be collected by DC using **SHINE\_Form 25\_v1 Birth visit**. Where notification of home birth is delayed (>7days) birth data will be transcribed by the DC from the Village Health Worker register. Table 7 outlines the specific types of data collected at each study visit. All interview data will be collected electronically and all biospecimens will be transported to a field laboratory for initial processing before being sent from the field to the study laboratory in Harare.

**Table 7 – Data and specimen collection from antenatal mothers**

<b><i>Antenatal Period</i></b>	
	<b>Study Visit</b>

	Baseline SHINE_ Form 13v5_ Baseline form	32 weeks Gestation SHINE_Form 15_v2_32 weeks Gestation	End of pregnancy SHINE_Form 14_v5_ End of Pregnancy form
<b>All Enrolled Mothers</b>			
Woman's vital status	x	x	x
Household composition	x		
Education, employment, child care	x		
IYCF practices for youngest child	x		
IYCF knowledge	x		
WASH knowledge	x	x	x
Exposure to intervention messages	x	x	
Use of health care	x		
Anthropometry of youngest child	x		
Woman's autonomy and social support	x		
Woman's physical and mental health	x		x
Mothering self-efficacy, time allocation and stress	x		
Household sanitation and hygiene reported practices and observations	x	x	x
Mother's chores and employment, household economic status, income and aid	x		
Tippy tap, soap, latrine, Water Guard knowledge and water chlorination test	x	x	
Socioeconomic status, aid, assets	x		
Post-harvest storage practices	x	x	
Household food security and dietary diversity	x		
Economic shocks	x		
Maternal height	x		
Maternal weight	x	x	x
Maternal mid-upper arm circumference	x	x	x
Maternal blood pressure	x	x	x
Mother's relationship to VHW	x	x	
<b>Pregnancy Assessment</b>			
Antenatal care of pregnancy	x	x	x
Past medical history	x		
7 day maternal health recall	x	x	x
Maternal 3 month symptom history	x	x	x
Pregnancy exposures (medication, alcohol, smoking)	x	x	x
Mother's reproductive health and previous pregnancy		x	
Delivery details			x
PMTCT history <sup>1</sup>			x
Maternal postnatal morbidity			x
Maternal clinic visit and hospitalization history			x

Infant birth details			X
Facility birth information			X
Infant 7 day symptom recall			X
Infant postnatal morbidity			X
Infant clinic visit and hospitalization history			X
Infant immunizations and vitamin A			X
Infant practices (fontanelle and cord care, malaria prevention)			X
Infant PMTCT (HIV-exposed infants only)			X
Miscarriage risk factors and post-miscarriage health <sup>2</sup>			X
Stillbirth verbal autopsy <sup>3</sup>			X
Infant verbal autopsy <sup>4</sup>			X
Maternal verbal autopsy <sup>5</sup>			X
<b>Maternal Biologic Specimen collection<sup>a</sup>::</b>			
L:M 2-hour urine collection	X		
Single sample urine collection	X	X	
Blood sample (for serum, plasma, cell storage)	X	X	
Saliva	X		
Stool	X		
Maternal HIV rapid test	X <sup>6</sup>	X <sup>6</sup>	
Maternal CD4 count	X <sup>7</sup>	X <sup>7</sup>	
Maternal Hemoglobin (Hemocue)	X		
Maternal urinary microscopy for schistosomiasis	X	X	
Maternal urinalysis	X	X	
<b>Paternal data collection</b>			
Paternal height <sup>8</sup>	(X)	(X)	(X)
Infant date of birth			X
Infant gender			X
Infant vital status			X
Infant birth weight			X
Infant birth length			X
IYCF practices for index child			X
Anthropometry of index child			X
Neonatal examination			X
Neonatal temperature and respiratory rate			X

<sup>1</sup>For HIV-positive mothers only<sup>2</sup>Only in women who have miscarriage (fetal loss before 28 gestational weeks)<sup>3</sup>Only in women who have stillbirth (delivery of a dead fetus >28 gestational weeks)<sup>4</sup>Only in women whose infant dies after a live birth<sup>5</sup>Only in cases of maternal death: consent for a verbal autopsy will be obtained from a close relative

<sup>6</sup> Women will be offered rapid testing in their home at baseline and again at 32 weeks (or those who were negative at baseline). HIV rapid testing will also be repeated in the laboratory using the same sample, to confirm the result obtained during the home visit. Women who prefer not to be tested in their home will be counseled on the benefits of learning their status during pregnancy and encouraged to be tested in the antenatal clinic. Blood samples collected from women who prefer not to be tested at home will be tested for HIV in the laboratory, but these results will be used only during analysis after personal identifiers are stripped from the HIV result data to interpret primary outcomes by maternal HIV status. If an indeterminate result is obtained in the laboratory, or if the result is different to the result obtained in the woman's home, women may be approached to provide a repeat blood sample so that definitive HIV status can be determined. If women request an HIV test at other study visits to learn their status, we will undertake a rapid test in the home where feasible.

<sup>7</sup>In HIV-positive women only

<sup>8</sup>Paternal height will be measured once during the course of the trial, whenever the father is available. He will be asked to provide consent to measure and record his height, using **SHINE\_Form 40v2\_Paternal Height Consent form**. This information will be used to calculate the mid-parental height, which is an important genetic determinant of growth


potential for the infant.

<sup>a</sup>Where specimens are not collected at a planned visit, either because the visit was missed or because it was not possible for another reason, those specimens will be collected as soon as possible thereafter, which may be outside the window of the original visit.

The mother's biospecimens will be used for the following assays:

#### Urine:

Lactulose:mannitol test: A lactulose and mannitol sugar solution will be ingested by women and urine collected for 2 hours at the 10-<26 weeks gestation baseline visit. A sample of urine will then be taken from the bottle, preservative added and the sample frozen for subsequent analysis of L:M ratio by mass spectrometry at Oregon Analytics, USA, and for long-term storage (see section 17).

Lactulose mannitol (L:M) solution will be made up in a clean bench-top hood in a dedicated preparation room at Zvitambo in Harare. Supplies will either be autoclaved prior to use (stir bars, spatula, beakers), or will be sterile (water, 50mL conical tubes).

20ml aliquots of L:M solution will be produced, using mannitol powder USP (Spect Chemicals and Laboratory Products, Gardena, CA, USA), lactulose solution USP (10g/15ml; Coghlan Group, USA) and sterile water, according to Table 8, below.

#### **Table 8 – Preparation of lactulose-mannitol solution**

20ml aliquots will be labeled with lot number, production date and expiry date (with 3 month shelf life) and stored in a dedicated refrigerator in Harare at 2-8C. A 5mL aliquot from every batch of L:M solution produced will be cryopreserved for any future QC testing.

A strict cold chain (2-8C) will be maintained while transporting aliquots of the L:M solution to the 4 field hubs, where they will be kept in a dedicated L:M refrigerator with 24hr back-up



generator. A log of refrigerated aliquots will be maintained by the laboratory technician at each site. Once a home visit requiring L:M administration is scheduled, the laboratory technician will label a vial of L:M solution to be used with a barcoded label that contains the study participant ID and date of visit.

Research Nurse Supervisors will dispense the L:M solution on the morning of the home visit, by matching the study subject ID and date with the barcoded vial, and DCs will transport it to the mother's house in a dedicated cold box with ice packs.

Upon arrival at the mother's home, the DC asks the mother to drink the whole 20ml dose. For infants, the DC will weigh the child, calculate the dose to be given (2ml/kg to a maximum of 20ml), measure the correct dose and give the solution with an oral syringe. The dose given will be recorded on the study form for that visit.

Single urine collection: A fresh sample of urine will also be collected from the pregnant women at both baseline and 32 weeks visits for urinary filtration and microscopy to detect ova of *Schistosoma haematobium*. Cryopreserved urine collected at the baseline visit from all enrolled women will be used to measure aflatoxin M1, which provides a measure of aflatoxin exposure over the prior 24-48 hrs, and will provide representative data on seasonality. Urinary creatinine will also be measured on these same women to correct for urinary dilution. Cryopreserved urine from both visits will be used to measure fumonisin (FB1), deoxynivalenol (DON and DON-G), T-2-toxin (T-2) (and its metabolites HT-2-toxin (HT-2), HT-2-toxin-4-glucuronide (HT-2-4-GlcA)), zearalenone (ZEA) (and its metabolites zearalanone (ZAN),  $\alpha$ -zearalanol ( $\alpha$ -ZEL), and  $\beta$ -zearalanol ( $\beta$ -ZEL) and corresponding 14-O-glucuronic acid conjugates (ZEA-14-GlcA, ZAN-14-GlcA,  $\alpha/\beta$ -ZEL-14-GlcA)) and ochratoxin A (OTA and ochratoxin alpha (OT $\alpha$ )), by LC/MS/MS in women enrolled in the preterm birth substudy. Cryopreserved urine from the baseline visit will be used to measure intestinal fatty acid binding protein (I-FABP), a marker of small intestinal damage, and results compared to plasma levels and L:M ratio.

Blood: (15 ml total)

**5ml** will be collected into two endotoxin-free EDTA tubes (10ml total). Plasma will be extracted and aliquots stored in endotoxin-free tubes at -80C for subsequent analysis of CRP, AGP, sCD14, lipopolysaccharide, I-FABP, ferritin, hepcidin, sTFR, aflatoxin-lysine and IGF-1,. The buffy coat layer will be removed, red cells lysed and white cells fixed for storage at -80C. Subsequent staining of thawed PBMCs with monoclonal antibodies will be undertaken to determine levels of T cell activation, senescence, differentiation and thymic output by flow cytometry. Thawed PBMCs or stored saliva can also be used to extract DNA subsequently. All enrolled women will be asked for informed consent for DNA extraction from stored samples, using **SHINE\_Form9v5Specimen Storage and Shipment Consent Form**. Women providing consent will be flagged in the database so that samples can be subsequently used for DNA extraction. Women who do not provide consent will be flagged in the database and aliquots labeled with different bar-codes so that samples are not subsequently used for DNA extraction, long-term storage or shipment. DNA would be used in future studies to investigate genetic determinants of infant health and growth.

For long-term storage plans see section 17.

**5ml** will be collected in a serum gel clot tube. Serum will be extracted and aliquots stored at -80C. For long-term storage plans see section 17.

A few drops (approx. 0.2ml) of blood will be taken from the second EDTA tube and transferred to the Hemacue cuvette for immediate hemoglobin testing and onto the HIV rapid test strip for immediate HIV testing.

**Saliva:**

Saliva will be collected by placing an oral swab under the tongue for 1-2 minutes. The whole swab is placed into a storage tube and frozen for subsequent analysis of salivary cortisol and cytokines (as listed under 'blood' above) and FUT2 ELISA to evaluate maternal capacity to fucosylate human milk oligosaccharides, which is linked to infant growth (Charbonneau *et al.*, 2016). Saliva could also be used as an alternative to PBMCs for DNA extraction in women who provide consent (see above). For long-term storage plans see section 17.

**Stool:**

Fresh stool will be collected into a container and 3 aliquots frozen for subsequent analysis of markers of enteric inflammation (fecal neopterin, alpha-1 antitrypsin, myeloperoxidase) and analysis of the intestinal microbiota. For microbiota studies, DNA and RNA will be extracted from approximately 220 mg of stool from each sample, using standard protocols (e.g., MoBio Laboratories; PowerFecal Microbiome RNA/DNA extraction kit). Paired-end libraries will be constructed using a non-commercial protocol for the Illumina HiSeq 2000 platform. RNA samples will be depleted of rRNA prior to sequencing. Sequencing will provide information about the total microbial diversity in the intestine (e.g., species richness and abundance) and the functions (e.g. resistance to pathogen colonization) carried out by the intestinal microbiota. In 150 mother-infant pairs, we will also define the metabolic activity (metabolomics) of the fecal microbiota at 32 weeks and 1 month post-partum for women (whose infants are in the EED substudy) and at 1, 3, 6, 12 and 18 months for infants, using an untargeted metabolomics approach by nuclear magnetic resonance spectroscopy.

Currently, there are no high throughput sequencing centers in Zimbabwe (or elsewhere in sub-Saharan Africa), or facilities to conduct nuclear magnetic resonance spectroscopy therefore stool (or the microbial DNA/RNA contained within these samples) will be transferred to Canada (University of British Columbia and Michael Smith Genome Sciences Centre, Vancouver, British Columbia, Canada) for sequencing and untargeted metabolomics. Samples transferred to Canada for sequencing and metabolomics will be identifiable by the study ID only. Chain of custody of the samples will be assured by a Zvitambo scientist who will accompany the samples to the collaborating laboratory. Samples will only be used for the analyses described in this proposal and remnants of samples will be destroyed after this work has been completed. Bioinformatics and biostatistical analysis will be carried out in conjunction with SHINE co-investigators and technical and bioinformatics training for Zimbabwean scientists will be encouraged as part of the analysis process.

For long-term storage plans see section 17.

**Maternal clinical assessment:**

DCs will follow SHINE clinical referral guidelines for any women with abnormal clinical signs or measurements. The DC will complete a SHINE clinical referral form and ask the mother to attend the clinic either routinely or urgently, according to the guidelines.

**End of pregnancy visit**

VHWs will report whether any pregnancies have ended (including miscarriage, stillbirth, live birth). In the event of a SHINE miscarriage or stillbirth, a Research Nurse Supervisor and DC will visit the mother together, within 2 weeks of the event being reported. The timing has been chosen to accommodate the sensitivity of this visit, which will include both a verbal autopsy regarding the fetal death, and completion of an Adverse Event form (see SAE section). For infants delivered at home the DC will conduct a visit to undertake anthropometry on the infant using **SHINE\_Form25\_v1\_Birth Visit**. For infants delivered in health institutions the DC will undertake the routine 1 month postnatal visit, using form

**SHINE\_Form 14\_v5\_End of Pregnancy form.** Subsequently visits will be conducted at 3 months postnatal using form **SHINE\_Form 20\_v4\_3 month visit**, at 6 months postnatal using form **SHINE\_Form 21\_v3\_6 months visit**, at 12 months postnatal using form **SHINE\_Form 22\_v2\_12 months visit**, and 18 months postnatal using **SHINE\_Form 23\_v2\_18 months visit**.

Table 9 specifies the components of each post-partum research data collection visit.

**Table 9 – Postnatal data and specimen collection schedule**

Study Visit									
All mother-infant pairs			Months Post-Partum						
	Birth <sup>1</sup>	1	3	6		12		18	24
Identification and Vital status of infant and mother	x	x	X	X		x		x	x
IYCF practice for index child		x	X	X		x		x	
Nutributter knowledge and use (IYCF arm only)						x		x	
Household sanitation and hygiene reported practices and observations		x	X	X		x		x	
Tippy tap, soap, latrine, Water Guard Knowledge (WASH only)		x	x	X		x		x	
Water chlorination test (WASH only)		x	X	X		X		x	
Play Mat Use (WASH only)				X		x		x	
IYCF and WASH Knowledge			X	X		x		x	
Exposure to intervention messages				X		x		x	
Immunizations and child health		x	X	X		x		x	
PMTCT and infant HIV diagnosis		X	X	X		X		X	
7 day maternal morbidity recall		x	X	X		X		X	
Maternal clinic visit and hospitalization		x	X	X		X		x	
Infant 7 day morbidity history recall		x	X	X		x		x	
Infant long-term illness recall		X	X	X		X		X	
Infant clinical examination		x	x	x		x		X	
Infant weight, length, head circumference and MUAC <sup>b</sup>	x	X	x	X		X		X	x
Assessment of early child development									x
Infant body composition, skinfold thicknesses, mid-thigh circumference and leg length									x
Mother's relationship to VHW			x			X		X	
Woman's autonomy, agency, social support				X				x	
Woman's physical and mental health				X				x	
Woman mental health (Edinburgh postnatal depression scale)		x		X		X			x
Woman's mothering self-efficacy, time, and stress				X				x	
Maternal weight and MUAC		x	x	x		x		x	

Mother's chores and employment; household socioeconomic status, income, and assets								x	
Post-harvest storage practices				x				x	
Household composition				x				x	
Education, employment, child care				X				x	
Market access and use of information				X				x	
Household food security and diversity				x				x	
Economic shocks				x				x	
<b>Specimen Collection</b>									
Infant blood sample <sup>2</sup>								X <sup>6</sup>	x
Woman's blood sample <sup>3</sup>								X <sup>6</sup>	
Paternal height <sup>4</sup>		x	x	x			x	x	
Infant saliva <sup>5</sup>									x

<sup>1</sup>For home deliveries only

<sup>2</sup>A 5.4 ml blood sample will be collected on all infants at 18 and 24 months by venipuncture, into two 2.7ml endotoxin-free EDTA tubes, using local anaesthetic cream for the blood draw if the mother wishes. A few drops of blood will be used to check hemoglobin using a Hemocue machine. If a mother is found to have become HIV-positive at the 18 month visit (ie seroconverted during the study), or already known to be HIV-positive, the infant blood will be used to test for HIV by DNA PCR and to measure CD4 count, to identify potential vertical transmission of HIV; the result of the infant HIV test will be returned to the mother by home visit. Whole blood will be stimulated with pathogen-associated molecular patterns in plates and incubated for 4-24hr. Supernatant will be removed and stored at -80C for subsequent analysis of pro- and anti-inflammatory cytokines, and cells will be fixed and stored for subsequent analysis of cellular activation, proliferation and cytokine elaboration by flow cytometry, to gain insights into functionality of the immune system. Remaining blood samples will be spun to collect plasma, and the cell pellet will be frozen. Blood will be stored according to the section on long-term storage plans and will be used for subsequent analyses of indicators of the EED pathway, as evaluated in the EED sub-study below, and to undertake hepatitis A serology, as an indicator of feco-oral exposure. The final choice of assays will be determined by the results of this sub-study. Consent will also be sought from mothers using **SHINE\_Form9v5\_ Storage and Shipment Consent Form** to subsequently extract DNA from the stored cell pellet for use in future studies to investigate genetic determinants of infant health and growth. Infants for whom consent is not given will be labeled with a separate bar-code and flagged in the database so that samples are not subsequently used for DNA extraction, long-term storage or shipment. Note that if an infant is in the EED sub-study, they should not have this blood sample taken, but instead should follow the schedule in Table 10.

<sup>3</sup>A 10 ml blood sample will be collected on all women at 18 months by venipuncture. HIV-negative women will be offered re-testing for HIV infection. We anticipate 0.8% HIV incidence per year among baseline HIV-negative women. Detection of HIV-infected women will enable timely referral for treatment, testing of infants who may be infected through breastfeeding for rapid referral to treatment, and reallocation of seroconverting women to the HIV-positive trial strata. As at previous testing opportunities, women who do not wish to learn their HIV status will be offered the option to have the HIV test conducted in the Zvitambo laboratory and not to receive the test result. Blood will also be stored according to the section on long-term storage plans and will potentially be used for subsequent analyses of indicators of the EED pathway. Woman who test HIV-positive at 18 months postnatal will have a CD4 count measured using the PIMA machine to assess their disease severity.

<sup>4</sup>Paternal height will be measured once during the course of the trial, whenever the father is available. He will be asked to provide consent to measure and record his height, using **SHINE\_Form**

**40v2\_Paternal Height Consent form.** This information will be used to calculate the mid-parental height, which is an important genetic determinant of growth potential for the infant.

<sup>5</sup> A saliva sample will be collected from the child at 24 months using a soft swab; samples will be stored at -80C for subsequent measurement of salivary cortisol.

<sup>6</sup> For 18 months visits that are conducted out of the study districts, where it will not be possible to process blood samples in the laboratory on the same day, a dried blood spot card will be collected and stored from infants instead of an EDTA blood sample. Mothers will be offered rapid HIV testing in the home but will not have an EDTA/serum blood sample collected.

<sup>a</sup> Where specimens are not collected at a planned visit, either because the visit was missed or because it was not possible for another reason, those specimens will be collected as soon as possible thereafter, which may be outside the window of the original visit.

<sup>b</sup> Anthropometry data and hemoglobin result at 18 months (primary trial endpoints) will be checked carefully by Research Nurse Supervisors at the SHINE hub; in cases where primary endpoints are either missing or outside the stipulated plausibility ranges, a home visit will be conducted to repeat the measurement(s).

## Infant Observation

In addition to the other data collection visits outlined above, we will conduct observation studies once on 180 mother-infant pairs during the course of the trial. These households will be selected by matching the gender and age of the youngest 90 infants in the WASH arms to 90 infants in the non-WASH arms. The purpose of these visits is to check compliance with WASH and IYCF behaviours, and therefore provide an additional in-depth measure of intervention uptake. A trained observer will conduct a 6-hour structured observation. The researcher will visit the mother and obtain consent using **SHINE\_Form 78\_v1\_Observation Study Consent Form**. After obtaining consent the researcher will observe the household as well as the normal daily routines of the mother using **SHINE\_Form 79\_v1\_Household and Baby Observation Form** to record specific baby activities and household observations.

## 18 month visit

An information sheet (SHINE Form 68\_v1\_Specimen and Testing non EED Information Sheet) which explains the reasons for specimen collection and haemoglobin measurement at 18 months will also be given to all participants, and women in the SOC and IYCF arms will also be provided with a SHINE Pledge Certificate v1 which pledges that a latrine will be built at the end of the trial.

In addition, informed consent will be sought to conduct an additional visit at 24mo of age using **SHINE\_Form 64\_v1\_Neurodevelopment at 24 Months Visit Consent Form**. The procedures for obtaining informed consent will follow those used for the main trial (i.e. if participants require time to consider and consult, the consent form will be left with them and a return visit scheduled for a later date).

## Assessment of Early Child Development

In the post-2015 era of the Sustainable Development Goals, there is a need to focus with renewed emphasis on whether children are thriving and whether their full human potential is being harnessed. This includes understanding whether they are developing well. The brain has the highest metabolic requirements of any organ, and stunting is closely associated with delayed developmental progression and later school outcomes. The precise links between stunting, “thrival” and neurodevelopmental outcomes have not been well explained.

Assessing child development allows us to evaluate more broadly whether SHINE interventions help children to ‘thrive’, beyond measures of illness and linear growth.

The caregivers of children who reach 24 months of age from February 2016 onwards (estimated 3200 total) will be invited to have an additional home visit to assess early child development (ECD). We will undertake an assessment of general child development in the areas of gross motor, fine motor, language and social behavior skills, and will also include more specific tests that would enable us to pick up more subtle changes in child cognitive function, emotional regulation and temperament (Table 3).

In order to develop and validate appropriate tools to broadly assess ECD in rural Zimbabwe, we will undertake the following series of steps to pilot test the procedures in the study context:

**Step 1:** we will individually interview 25-30 mothers of non-SHINE children age 18 to 30 months seeking information concerning the words and grammatical markers (such as pre-fixes) their children use using **SHINE\_Form 61\_v1\_MacArthur Bates Pilot 18-30 months Data Collection Form**. We will seek written informed consent from caregivers at local clinics to undertake the interview (which will last 30-60 minutes), using **SHINE\_Form 62\_v1\_MacArthur Bates Language Development Pilot 18-30 months Consent Form**. From these interviews, we will develop a list of at least 400 words for piloting, as well as an appropriate number of grammatical markers.

**Step 2:** We will pilot test the list of words, grammatical markers and/or word combining items, plus words that span a range from easy to more difficult, with a second cohort of 100 mothers of non-SHINE children age 18-30 months. We will also ask these mothers if their children say any words (or pre-fixes) that are not on the list. We will seek consent from caregivers at local clinics to undertake the interview (which will last 30-60 minutes), using **SHINE\_Form 62\_v1\_MacArthur Bates Language Development Pilot 18-30 months Consent Form**.

**Step 3:** We will pilot test all the SHINE early child development tests with up to 20 children, using **SHINE\_Form 63\_v4\_24 month visit data collection form**. These 20 children will be drawn from the SHINE cohort, but their scores will not be used in the final dataset and these children will not subsequently be included in the 24 month evaluations. Caregivers of these children will be approached to provide consent using **SHINE\_Form 64\_v1 Neurodevelopment at 24 months Pilot Consent Form**.

Following these piloting steps, translated forms for the ECD tests being used will be finalized by amending **SHINE\_Form 63\_v4\_24 month visit data collection form**. Periodically, and particularly after initial training, the administration of tests by Data Collectors will be video recorded so that an independent assessor can validate the assessments, thereby facilitating their standardization assessments. Permission for video recording will be sought from caregivers using **SHINE\_Form 66\_v1 Video Neurodevelopment at 24 months Consent Form**. These permissions will include optional consent for the storage of video recordings for teaching and presentation purposes; otherwise, recordings will be destroyed after review and analysis as specified in the consent form.

The 24 month visit will be conducted by DCs using **SHINE\_Form 63\_v5\_24 month visit data collection form**. Consent will be sought using **SHINE\_Form 65\_v3 Neurodevelopment at 24 months Consent Form**. In the case of interviewing a guardian instead of a biological mother, **SHINE\_Form 72\_v1\_Guardian Neurodevelopment at 24 months visit Consent** will be used; this will be in situations where the mother has died or moved away and the primary caregiver is an extended family

member. Consent for Shipment and Storage of samples will be sought using **SHINE\_FORM 73\_v1\_Specimen Storage and Shipment at 24 months visit Consent**. In the case of a guardian instead of a biological mother consent will be sought using **SHINE\_Form 74\_v1\_Guardian Specimen Storage and Shipment at 24 months visit Consent**. The visit will be conducted among infants who have moved from their primary SHINE home but are still available to be visited within the study districts.

Caregivers of children identified as having developmental delay will be provided with some written information SHINE\_Form 67\_v1\_Parent Information sheet about how to stimulate the child to encourage developmental potential. This information will be provided in training by Dr. Melissa Gladstone (trainer) to all DCs and will be based on the UNICEF/WHO Care for Child Development intervention programme ([http://www.unicef.org/earlychildhood/index\\_68195.html](http://www.unicef.org/earlychildhood/index_68195.html)). Children with substantial developmental delay (defined as delay in more than 3 items in two areas of development on the Malawi Developmental Assessment Tool) or obvious disability (as identified in the child functioning and disability module) will be referred to local health facilities using a SHINE clinical referral form. We will work with the district therapists during the piloting stages to develop appropriate referral pathways.

### ***Body composition at 24 month visit***

At the 24 month visit, we will also more fully evaluate the impact of the randomized trial interventions on body composition, in a subgroup of children turning 24 months from September 2016. We will undertake bioelectrical impedance vector analysis, using a BodyStat 1500MDD machine, which uses an undetectable current measured through electrodes attached to the limbs to assess lean and fat mass. We will measure skinfold thicknesses using Holtain calipers to assess subcutaneous fat, mid-calf circumference and tibial length using a handheld knemometer, which non-invasively measures the distance from knee to ankle and provides a better measure of long-term metabolic health associated with stunting. Consent will be obtained for these additional measurements using **SHINE\_Form 70\_v2 Neurodevelopment and Body Composition at 24 months visit Consent Form** and data collected using **SHINE\_Form 71\_v3 Body Composition Data Collection Form**.

### **Environmental Enteric Dysfunction (EED) Sub-study**

Selection: All women who enrolled in the trial from 1<sup>st</sup> May 2014 onwards will be invited to join the EED substudy at the 32 week gestation visit, or as soon as possible thereafter. All dyads where the mother is HIV-positive (approximately 600) will be invited to join to the study.

Where a mother has refused to learn her HIV test result, she will not be eligible for the EED sub-study.

#### Informed Consent for EED Sub-study:

At the 32 week visit, or as soon as possible thereafter, the DC will discuss the substudy with all women, and provide them with the EED Sub-Study Consent Forms (**SHINE\_Form7\_v8\_EED Sub-study Consent Form; SHINE\_Form8\_v8\_EED Sub-study Consent Form**). Two consent forms are available – one for HIV-negative mothers and one for HIV-positive mothers. The only difference in procedures is that blood samples collected from HIV-exposed infants will be tested for HIV by DNA PCR for diagnostic purposes so that HIV-positive infants who become infected can be rapidly referred for treatment. NOTE: Zimbabwean government health facilities routinely test HIV-exposed infants at 4-6 weeks

and again 1 month after breastfeeding ceases, so infants enrolled in the trial will receive more testing and referral services than available through the public sector health services.

Mothers who feel ready to consent to the EED substudy after reading the consent form can provide their consent at the 32 week visit; women who want more time to discuss the substudy will be given the consent form to keep and will be asked again for their consent at the 1 month postnatal visit. Women who missed the 32 week visit and who are approached to enroll in the EED substudy at a subsequent visit can provide consent at that visit if they feel ready to, or else a follow-up EED consent visit will be made once the mother has had time to consider the substudy. From mother-infant pairs enrolled in the EED Substudy detailed morbidity data will be collected. To assist in collecting these data, EED Substudy mothers will be given a cell phone and solar charger, with airtime topped up at each research visit, as described in the consent forms (**SHINE\_Form7\_v8\_EED Sub-study Consent Form; SHINE\_Form8\_v8\_EED Sub-study Consent Form**).

Any HIV-positive mothers who gave birth prior to the launch of the EED substudy will be approached to give consent for the EED substudy at subsequent visits (ie beyond 32 weeks gestation) to ensure that all HIV-exposed infants are enrolled and final HIV status ascertained. This is important both for stratification of infants for the primary outcome, and to ensure that any HIV-infected infants are identified early and access immediate ART.

**Data Collection for EED Sub-study:** The schedule for additional data collection beyond that for the main trial is listed in Table 10. The EED substudy ends at the 18 month (primary endpoint) visit. No EED samples are collected at the 24 month visit. EED infants at the 24 month visit have data and specimens collected as outlined previously in Table 10. Mothers will be provided with an information sheet (SHINE Form 69\_v1\_Specimen and Testing EED Information Sheet) which explains the reasons for specimen collection and haemoglobin testing at each visit.

**Table 10 – Data and sample collection from EED substudy participants**

<b>Additional data for EED Sub-study Infants</b> <i>[N=1000 non-HIV exposed (250/grp); N~600 HIV-exposed]]</i>	Antenatal	Postnatal				
	30-34 weeks	1	3	6	12	18 <sup>1</sup>
Informed Consent for EED Sub-study	x	(x) <sup>1</sup>	(x) <sup>2</sup>	(x) <sup>2</sup>	(x) <sup>2</sup>	(x) <sup>2</sup>
Intensive 24 hour dietary recall (50 infants per arm only)					x	
Infant pre-LM single urine sample			x	x	x	x
Infant Lactulose:Mannitol test (2 hour urine collection)			x	x	x	x
Infant stool collection		x	x	x	x	x
Infant blood draw (2.5 ml in Paxgene tube, 2.7 ml in EDTA tube) <sup>3</sup>		x	x	x	x	x
Infant saliva collection			x	x	x	x
Infant rapid hemoglobin test			x	x	x	x
Infant HIV testing by DNA PCR <sup>4</sup>		x	x	x	x	x
Infant CD4 count <sup>5</sup>		x	x	x	x	x
Infant morbidity diary (continuous)						



Infant diarrhoeal stool collection (continuous, during diarrhoeal illness only)						
Maternal blood sample		x				
Maternal Stool sample		x				

<sup>a</sup>When an infant is in the EED substudy, specimen collection at 18 months should follow this table, and not the specimen collection schedule for infants in the main trial (Table 9)

<sup>1</sup>For 18 month visits that are conducted out of the study district, where it will not be possible to process samples in the laboratory on the same day, a dried spot card will be collected and stored from infants instead of an EDTA blood sample. Pre-LM and LM Urine, stool and saliva will not be collected on infants on long-distance visits.

<sup>2</sup>If not consented at the 32 week gestation visit

<sup>3</sup>Local anaesthetic cream can be used prior to venipuncture if the mother wishes.

<sup>4</sup>Infants born to HIV-positive mothers only; a dried blood spot will be made in the field laboratory and transported to the Harare laboratory for PCR. Results will be returned to the mother by home visit. If the sample result is indeterminate, we will conduct a home visit to give the result to the mother and request a repeat infant blood sample to clarify his/her HIV status. The repeat sample will be sent to the Harare laboratory for HIV DNA PCR and the result returned to the mother by home visit.

<sup>5</sup>HIV-exposed infants only

Where specimens are not collected at a planned visit, either because the visit was missed or because it was not possible for another reason, those specimens will be collected as soon as possible thereafter, which may be outside the window of the original visit.

#### Infant morbidity diary

Mothers of infants in the EED Sub-study will be asked to keep a daily morbidity diary (SHINE Form\_26v2) using a simple calendar. For each day, the mother will be asked to place a sticker with pictures on the calendar of the symptoms experienced by the infant on that day. A laminated booklet with pictures explaining each symptom below will be left with the mother:

**Table 11: Morbidity diary for infants in EED substudy**

Symptom	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Diarrhoea (3+ loose water stools per day)							
Blood or mucus in stool							
Hot body							
Cough							
Difficult or fast breathing							
Inability to feed or lethargy							
Took child for care							

To assist the mother in learning to complete the form, a SHINE field worker may make home visits to the mother during the first few months, to collect the completed diary, give a new one for the subsequent period, and encourage and thank the mother for her effort in keeping this diary. Women with adequate cellphone coverage may be supported by reminder SMS and/or phone calls to collect morbidity data instead of home visits.

To determine which supportive method (SMS, phone call or home visit) is most effective in collection of morbidity data, the first 40 women enrolled to the EED substudy will be part of a pilot evaluation: 10 women (Group 1) will be given a morbidity diary and will receive supportive home visits 2 weeks and 6 weeks after being given the diary; 10 women (Group 2) will not receive a diary but will be contacted once per week by phone to collect morbidity data and visited once at 6 weeks; 10 women (Group 3) will be given a morbidity diary and additionally receive a weekly phone call to collect morbidity data and will be visited once at 6 weeks; and 10 women (Group 4) will be given a morbidity diary and will receive supportive home visits 2 weeks and 6 weeks after being given the diary plus a weekly SMS reminder to complete the diary. At the 6 week visit, women in all groups will be asked to evaluate their experience in recalling morbidity data using **SHINE\_Form\_44v1\_Morbidity Diary Pilot Evaluation Form**.

#### Diarrhoea specimen collection

Women in the EED substudy enrolling from June 1st 2015 onwards will be asked to use the cellphone provided to call the SHINE hub whenever their infant develops diarrhoea (defined as  $\geq 3$  loose watery stools per day) or blood/mucus in the infant stools so that a Data Collector can make a home visit to collect a stool specimen. The DC will assess the infant during the home visit for dehydration and recommend a clinic visit if necessary. Stool samples will be frozen at -80C for subsequent analysis of enteric pathogens by PCR using a TaqMan array card assay at the University of Virginia, USA (Liu *et al.*, 2014), since the appropriate PCR platform is not available in Zimbabwe. Consent for this sample collection will be obtained at the 3 month postnatal visit, or as soon as possible thereafter, using **SHINE\_Form 50v1 Diarrhoea Sample Collection Consent**, and when diarrhoeal specimens are collected, **SHINE\_Form 51v3 Diarrhoea Sub-study Visit** will be completed. Mothers in the diarrhea substudy will receive a phone call every 2 weeks to remind them to collect a specimen if the infant develops diarrhea. Mothers will also be provided with SHINE\_Form 75\_v1\_Diarrhea Sample Collection Information Sheet to provide further information about the diarrhea substudy.

#### Intensive dietary recall

The mothers of 50 randomly selected infants per arm (total sample size = 200) in the EED substudy will be asked a set of intensive dietary recall questions covering the prior 24 hours at the 12mo postnatal visit, using **SHINE Form 43v3 24Hr Dietary Recall Form**.

Following consent (**SHINE\_Form10\_v5\_Specimen Storage and Shipment EED Infant Consent Form**) infant EED Sub-study biospecimens will be used for the following assays:

#### Urine:

Infant lactulose:mannitol test: A pre-L:M urine sample will be collected from the baby, then lactulose and mannitol sugar solution will be ingested by infants and post L:M urine collected for 2 hours. A sample of urine will then be taken from the bottle, preservative added and the sample frozen for subsequent analysis of L:M ratio by mass spectrometry at Oregon Analytics, USA, and for long-term storage (see section 17). Cryopreserved urine will be used to measure intestinal fatty acid binding protein (I-FABP), a marker of small intestinal damage, and results compared to plasma levels. Urine from 6,12 and 18 mo time-points in 1600 enrolled in the EED sub-study will be used to measure fumonisin, DON, T-2-toxin (T-2) (and its metabolites HT-2-toxin (HT-2), HT-2-toxin-4-glucuronide (HT-2-4-GlcA)), zearalenone (ZEA) (and its metabolites zearalanone (ZAN),  $\alpha$ -zearalanol ( $\alpha$ -ZEL), and  $\beta$ -zearalanol ( $\beta$ -ZEL) and corresponding 14-O-glucuronic acid conjugates (ZEA-14-GlcA, ZAN-14-GlcA,  $\alpha/\beta$ -ZEL-14-GlcA)) and ochratoxin A (OTA and ochratoxin alpha (OT $\alpha$ )), by LC/MS/MS. In a group of 1000 infants (500 with 'good growth', defined as LAZ>-0.5 at 18

months; and 500 with 'poor growth', defined as LAZ<-2 at 18 months) we will send urine samples from the 12 month visit to Dr Jon Swann at Imperial College London to undertake untargeted metabolomics by NMR and/or mass spectrometry, which are not available in Zimbabwe. Metabolomics is a powerful technology for assessing metabolic pathways without a priori hypotheses, which will provide unique insights into the pathogenesis of stunting beyond the EED pathway that we are already testing.

#### Blood (5.2ml total at each visit)

2.7 ml will be collected at each time point into an endotoxin-free EDTA tube. Plasma will be extracted and aliquots stored at -80C for subsequent analysis of CRP, sCD14, ferritin, hepcidin, sTFR, IGF-1, , anti-rotavirus IgA, anti-poliovirus IgA (serotypes 1, 2 and 3), anti-tetanus IgG, aflatoxin B1-lysine and collagen X. The anti-rotavirus IgA assays will be conducted at the University of Vermont, USA, on shipped plasma samples because the methodology is not able to be established in country. Plasma will be sent to Imperial College London to measure citrulline, kynurenine:tryptophan ratio (plus metabolites along the tryptophan/IDO1 pathway, ) and essential amino acids by mass spectrometry. The buffy coat layer will be removed, red cells lysed and white cells fixed for storage at -80C. Subsequent staining of thawed PBMCs with monoclonal antibodies will be undertaken to determine levels of T cell activation, senescence, differentiation and thymic output by flow cytometry.

2.5ml will be collected at each time point into a PAXgene Blood RNA tube. This tube contains a preservative that stabilizes RNA and allows subsequent analysis of gene expression profiles, to explore mechanistic pathways underlying stunting in a non-biased way. Tubes are stored at -80C until extraction of RNA. For long-term storage plans see section 17.

At the one month visit, DCs will encourage HIV-positive mothers to have their babies tested for HIV at 6 weeks through the early infant diagnosis (EID) service of the government PMTCT program and to initiate cotrimoxazole prophylaxis. In addition, the SHINE laboratory will test all HIV-exposed infants enrolled in the trial at 1, 3, 6, 12, 18 and 24 months by HIV DNA PCR on dried blood spot to ensure early diagnosis of HIV among breastfeeding infants. Any positive HIV DNA PCR results will be reported back to the family by the DC within 4 weeks, and referral to the local clinic made to access treatment. Thus, infants participating in the trial will benefit from more testing and referral opportunities than routinely available through public sector health services.

#### Saliva:

Saliva will be collected by placing an oral swab under the tongue for 1-2 minutes. The whole swab is placed into a storage tube and frozen for subsequent analysis of salivary cortisol and FUT2 ("secretor status") which is important in modulating the response to epithelial injury and is a determinant of intrinsic susceptibility or resistance to certain enteric viral infections, such as norovirus and rotavirus; maternal-infant secretor status therefore an important potential effect modifier in SHINE. For long-term storage plans see section 17.

#### Stool:

An aliquot of fresh stool will be collected into a container and frozen for subsequent analysis of markers of enteric inflammation and repair (fecal neopterin, alpha-1 antitrypsin,

myeloperoxidase and REG1), enteropathogen carriage by Taqman Array Card (conducted at the University of Virginia) and intestinal microbiota (as for maternal samples; see page 55 for details). In 150 mother-infant pairs, we will define the metabolic activity (metabolomics) of the fecal microbiota at 1, 3, 6, 12 and 18 months (as for maternal samples; see page 55 for details). For long-term storage plans see section 17. Though the original consent form stated that infant stool would be examined for soil transmitted helminthes, subsequent information from a national survey documented that the prevalence of STH among school age children was 1.5% in Shurugwi and 0% in Chirumanzu. Thus, finding any STH infection in 18 month old children is extremely unlikely. Accordingly, the research nurse will inform the mother at the 18 month visit that we are no longer going to test the baby's stool for helminthes because a large survey by the Ministry of Health and Child Care determined this is not a problem among children living in Shurugwi and Chirumanzu. Schistosomiasis will not be screened for routinely in children at 18mo because infection is associated with water-related activities in older children, and there are no documented safety data regarding praziquantel in children <4 years of age.

#### Maternal samples in EED substudy:

At 1mo postnatal, paired maternal blood and stool will be collected, to enable evaluation of mother-infant pairs soon after birth. 5ml of blood will be collected into an EDTA tube; plasma and PBMC will be extracted and stored, to enable comparison of maternal and infant biomarkers (CRP, sCD14, plasma multiplex cytokines, , lipopolysaccharide, IGF-1, ) and flow cytometry panels. If maternal HIV status was not confirmed using samples collected at baseline and/or 32 weeks, HIV testing will be repeated using this sample to ensure final maternal HIV status has been determined, as stated in the main trial consent form. **5ml** of blood will be collected in a serum gel clot tube. Serum will be extracted and aliquots stored at -80C. For long-term storage plans see section 17. Maternal stool will be collected and aliquots stored at -20C for paired analysis of maternal and infant microbiota, using methods as described on p43.

#### Infant clinical assessment:

At each visit, infants will be assessed by the DC for acute illness according to IMCI (2005) guidelines. DCs will follow SHINE clinical referral guidelines for any infants with abnormal clinical signs or measurements. The DC will complete a SHINE clinical referral form and ask the mother to take the child to clinic either routinely or urgently, according to the guidelines.

#### **Risk Factor for Preterm Birth Sub-Study**

The mother-infant dyads selected into this case control study will be identified prospectively as infants are born. Maternal baseline samples of blood, stool, and urine will be drawn from the archive for analysis as the case and control infants are identified. The informed consent form for the overall trial includes these investigations; therefore no additional consent form will be used for this study.

#### **Risk Factors for Stillbirth Sub-Study**

The mother-infant dyads selected into this case control study will be identified prospectively as infants are born. Maternal baseline samples of blood, stool, and urine will be drawn from the archive for analysis as the case and control infants are identified. The informed consent form for the overall trial includes these investigations; therefore no additional consent form will be used for this study.

#### **Risk Factors for Miscarriage Sub-Study**

The mothers selected into this case control study will be identified prospectively as miscarriages are reported. Maternal baseline samples of blood, stool, and urine will be drawn from the archive for analysis as the cases and controls are identified. The informed

consent form for the overall trial includes these investigations; therefore no additional consent form will be used for this study.

### **Risk Factors for Neonatal Death Sub-Study**

The mother-infant dyads selected into this case control study will be identified prospectively as infants are born and undergo the End of Pregnancy (1mo postnatal visit). Maternal baseline samples of blood, stool, and urine will be drawn from the archive for analysis as the case and control infants are identified. The informed consent form for the overall trial includes these investigations; therefore no additional consent form will be used for this study.

### ***Number of study contacts or visits required of participants***

Research Staff: Each mother will be seen once or twice to seek consent for the trial, twice during pregnancy, 5-6 times post-partum for data collection (at birth (for home deliveries only) using the **SHINE\_Form25\_v1\_Birth Visit** to record birth anthropometry; at 1 month using form **SHINE\_Form 14\_v5\_End of Pregnancy form**; at 3 months postnatal using form **SHINE\_Form 20\_v4\_3 month visit**, at 6 months postnatal using form **SHINE\_Form 21\_v3\_6 months visit**, at 12 months postnatal using form **SHINE\_Form 22\_v2\_12 months visit**, and 18 months postnatal using **SHINE\_Form 23\_v2\_18 months visit**; plus 2 brief morbidity diary visits using the **SHINE\_Form 26\_v2\_Morbidity diary** during month 2 and one extra diary visit during month 3 for a total of 11-13 contacts. All infants turning 24 months of age from February 2016 onwards will additionally have a visit using **SHINE\_Form 63 v5\_24 month visit data collection form**. All post-partum visits will be to the mother-infant dyad.

Village Health Workers: Each mother will be seen at least three times during pregnancy and at least monthly between delivery and 18 months post partum.

### **Maternal or infant deaths**

VHWs will report maternal or infant deaths in accordance with their regular reporting. A Research Nurse Supervisor and DC will visit the mother together, within 2 weeks of being informed of the event. The timing has been chosen to accommodate the sensitivity of this visit, which will include both a verbal autopsy regarding the maternal or infant death. Maternal verbal autopsies will be conducted with a family member using SHINE Form 35v1 Maternal Verbal Autopsy; infant verbal autopsies will be conducted with the mother, or with another family member in the case of maternal deaths using SHINE Form 13v4 (End of Pregnancy Form) for infants <1 mo old at the time of death, and SHINE Form 45v1 Infant Verbal Autopsy for infants >1 mo old at the time of death. In all cases of maternal or infant death, the study team will also undertake completion of an Adverse Event form (see SAE section).

### **Maternal or infant hospitalizations**

VHWs will report maternal or infant hospitalizations (defined as an overnight stay in a medical facility). This will trigger an SAE visit within 2 weeks of being informed of the event.

### ***Expected duration of the study***

For each mother, we expect to enroll by 24 weeks gestation and follow her until her baby is 18 months old. Thus each mother will participate for up to 25 months. The entire study (from enrollment of the first pregnant mother to the 18 month visit of the last enrolled mother) will run an estimated 3 calendar years. We anticipate at least 2 additional years for laboratory and data analysis. From February 2016, infants will be assessed during one additional visit at 24 months of age.

### ***Data Analysis***

#### ***Summary of Analytic Approaches for SHINE Outcomes***

As a cluster randomized trial, SHINE outcomes, in general, will be analyzed using statistical methods that account for within-cluster correlation. With a relatively large number of clusters (212), and for some analyses, only a few events or prevalent cases per cluster, generalized estimating equations (GEE) estimation provides an ideal approach to data analysis. We will use the Stata GEE regression procedure, with exchangeable correlation structure within each cluster, for both continuous and discrete response variables. Primary analyses will contain two dummy variables representing each intervention in this 2x2 factorial study; they will be unadjusted for other covariates. A log link function will be used for prevalence outcomes, to aid in interpretability by directly producing risk ratio estimates. Although this study is not powered to detect a statistical interaction effect between the two interventions, it will be estimated. If there is an important interaction, we will also present results using a regression model with three terms to represent the four study arms. The case-control sub-studies will be analyzed via conditional logistic regression models. Because of the conditioning on the case-control sets, and the fact there will usually only be one case per cluster, there will be little opportunity for intra-cluster correlation to be exhibited.

### ***Screening for Eligibility and Assignment to Study Groups***

All women who become pregnant and are identified by the Village Health Worker during their pregnancy surveillance activities are eligible for the trial. A research nurse will visit and request her participation during the informed consent visit. Assignment to treatment group is based on the randomization of the cluster and the research nurse will have no involvement in this process.

### ***Masking***

Due to the types of interventions being tested in this trial, masking of either the participants or investigators is not possible. However, we have specifically chosen a cluster randomized design and a separation of the intervention and data collection teams to reduce the potential for bias as much as possible.

### ***Receipt of Routine Care***

All subjects will continue to receive routine care through the government or other health care system. There will be no interference with access to care. In fact, participation in this trial elevates the routine care available to all participants as we have conducted extensive health system strengthening prior to beginning this trial and research staff will promote appropriate health care attendance.

### ***Use of Placebo or Non-Treatment Group***

No placebo will be used for this trial, but there will be a control group assigned to neither of the intervention arms. The justification for withholding these interventions from the control group is that we still don't fully understand the separate and combined effects of these interventions on the growth of young children. All participating households will receive a latrine or upgrades to bring a currently owned latrine to MOHCC standard, once the trial period is completed.

### ***Describe what happens to participants receiving therapy when the study ends or if a subject's participation ends prematurely***

The trial will not interfere with a participant's access to care at any time during the study or after the trial ends.

***Referring subjects to care outside the study***

The data collectors are qualified primary care nurses who will refer participants for care to the appropriate level of the health system based on their professional judgment at the time of each study visit. Village Health Workers are part of the health care system and will refer as well based on their training and the protocols and policies of the MOHCC.

***Loss to follow up***

For each scheduled intervention visit, 2 attempts will be made by a VHW to deliver the appropriate module; if the mother was not available on either occasion then this will be considered a missed module. Regardless of intervention delivery, the modified intent-to-treat analysis will include all infants who enrolled and provided data on length and hemoglobin at the 18 month endpoint. Data on module delivery will be collected for the per-protocol analysis, which in part will be based on successful arm-specific module delivery after 24 gestational weeks. For each research visit, the DC will make at least 2 attempts to visit the woman's original home; if feasible, field staff will make additional attempts to make a visit within the allowable window. If data cannot be collected, it will be considered a missed visit. Women who move to a new home may be visited by a VHW at their discretion to receive modules but will not receive any intervention inputs: Nutributter (IYCF arms) or latrine, Tippy Taps, soap, Water Guard and infant play space (WASH arms) at their new home. Research visits will not be conducted on mother-infant pairs who move from their original home, except at 18 months, when a final attempt will be made to collect primary endpoint data (height and hemoglobin) in their new location. If women return to their primary home, intervention delivery will resume, and will catch up where hardware inputs (e.g. latrine, Tippy Tap) have been missed (except Nutributter). At each time point, data will be collected on time spent away from home (defined as an overnight stay) since the last DC visit and a cumulative total will be calculated over the course of the trial, to be used in per-protocol analyses.

If study subjects wish to withdraw from the trial, the SHINE Exit Form (**SHINE\_Form 24\_v3\_Exit Form**) will be completed by the Research Nurse Supervisor. Women who withdraw from the trial will no longer receive any study inputs, including a latrine at the end of the trial; VHWs may resume routine MoHCC activities. Study participants who have withdrawn but wish to resume their participation in the study can contact the SHINE team and provide informed written "reconsent" (using intervention arm specific forms, **SHINE\_Form 57, 58, 59 and 60v1\_Reconsent Form**).

***Power Calculations***

See previous sections for the main trial and sub-studies for these calculations.

***Diagnostic tests***

A number of diagnostic tests will be provided to participants in this trial. This includes HIV testing for mothers and infants, hemoglobin testing for mothers and infants, urine-based assays for schistosomiasis and dip stick urinalysis in mothers, and two blood pressure measurements in mothers at baseline and 32 weeks gestation. All other tests are research oriented and do not currently have clinical usefulness in this setting.

**HIV Test:** Women and infants will be tested for HIV according to the Zimbabwean MOHCC National HIV-1 Testing Algorithm. For women, an anti-HIV antibody rapid test (Alere Determine HIV 1/2; sensitivity 99.9%, specificity 98.2%) will be undertaken in the woman's home, or in the laboratory if she prefers. If negative, she will be considered HIV uninfected. If positive, a second rapid test (INSTI HIV-1/HIV-2 Rapid Antibody Test; sensitivity >99.8%, specificity >99.5%) will be undertaken immediately. If positive, she will be considered HIV-

infected. In the case of discordant results, both rapid tests will be repeated. If both remain discordant, HIV testing will be repeated using HIV DNA PCR in the central laboratory. Infants will have blood collected onto filter paper and the dried blood spot will be tested by HIV DNA PCR at 3, 6, 12 and 18 months (Roche Amplicor HIV-1 DNA test v1.5; sensitivity 100%, specificity 100%) (Zijenah 1999).

**Hemoglobin testing:** Maternal and infant hemoglobin will be measured on whole blood using a HemoCue assay. Mothers with moderate anemia (Hb<100 g/L) will be referred to the local clinic, as per Zimbabwean antenatal guidelines. Infants with severe anemia (hemoglobin <70 g/L) or moderate anemia (hemoglobin <110 g/L) with clinical symptoms will be referred for work-up and care to the local clinic.

**Urine Schistosomiasis Assay:** The classic urinary filtration method will be used for diagnosis, as originally described by Mott(1982) and widely used by field clinicians. Presence of microhematuria by dipstick testing will also be assessed. Although there is no 'gold standard' test for diagnosis of urinary schistosomiasis, microscopy was found to be the best of five diagnostic methods evaluated (microscopy, urinary antigen testing, serology, ultrasound or detection of hematuria) in Ghana, with sensitivity of 82-100% and specificity of 93-98%, depending on age and geographical location (Mott 1982, Koukounari 2009). A 10ml aliquot of maternal urine will be syringe filtered through a membrane and the filter examined for eggs of *Schistosoma haematobium*. Results will be expressed as eggs per 10mL urine.

**Blood pressure (BP):** BP will be measured in pregnant women at the two antenatal visits (10-<20 weeks and 32 weeks gestation) using an ADC Advantage 6021 Automatic Electronic Blood Pressure Monitor. Women will be referred to the local clinic, as per Standard Treatment Guidelines for Zimbabwe (EDLIZ) 2011, if they have evidence of: i) a rise in diastolic blood pressure >15mmHg between visits; ii) mild pregnancy-induced hypertension (PIH), ie diastolic BP 90-100mmHg with no proteinuria; iii) moderate PIH, ie diastolic BP 100-110mmHg with no proteinuria; iv) severe PIH, ie diastolic BP >110 mmHg with no proteinuria; v) pre-eclampsic toxemia, ie any of i), ii) or iii) in the presence of proteinuria; vi) imminent eclampsia, ie proteinuria and hypertension with symptoms of visual disturbance or epigastric pain; and vii) eclampsia, ie pregnancy-induced hypertension with seizures.

**Urinalysis:** Urinalysis will be undertaken in pregnant women at the two antenatal visits (10-<24 weeks and 32 weeks gestation) using Bayer Multistix 10 SG Reagent Strips, which measure bilirubin, blood, glucose, ketones, leukocytes, nitrites, pH, protein, specific gravity and urobilinogen. If there is evidence of glycosuria, proteinuria, urinary tract infection (positive leukocytes and/or nitrites), or bilirubinuria, women will be referred to the local clinic.

**Temperature:** Axillary temperature will be measured in children using a single-use, sterile clinical thermometer (3M Tempa-DOT™) according to the manufacturer's instructions. These are widely used in routine clinical practice, have a sensitivity of 100% and 95.4%, respectively, in children and have been shown to be a reliable alternative to the mercury thermometer in validation studies (van den Bruel 2005).

## Study Procedures in Sequential Order for Village Health Workers

All Village Health Workers operational in the two districts will be approached to provide informed consent for research data collection (**SHINE\_Form 11\_v3\_VHW\_Consent\_Form**)



Consenting Village Health Workers will be contacted up to 10 times for research data collection during the course of the study – over a 5-year period. On 5 of these visits (at intervals of one year, from the study's onset) questionnaires (**SHINE\_Form 12\_v3\_VHW\_Questionnaire**) will be administered to ascertain Village Health Workers work capacity, performance as well as the motivational and supervisory characteristics of their work. Village Health Workers will also undergo semi-structured observations (shadowing) for up to 4 times during their normal activities (e.g. home visits, intervention delivery visits, report writing, and travelling) to further assess their work performance and establish the fidelity of delivery of study interventions.

## 5. Data Security and Protection of Subject Confidentiality

Prior to data collection all Research Staff will be trained on the protection of human subjects in a 3-day course taught by the Medical Research Council of Zimbabwe staff (MRCZ, the local IRB). Key Zvitambo research staff have previously received local training, facilitated by the MRCZ, on Basic Health Research Ethics and Basic Good Clinical Practice (GCP).

### a. Applying for a Certificate of Confidentiality?

No

### b. Data security plan

Hard Copy of data collection form: Indicate your choice but typing an X in the appropriate box on the left:	
X	Hard copies of data collection materials <u>have identifiers</u> and are locked in a secure cabinet or room with limited access by specified individuals. When possible, redacted (de-identified) versions of the data collection sheets will be used for coding and analysis. <b>NOTE:</b> Paper versions of all forms will be available for use when electronic devices fail for any reason. Paper forms will also be used for administrative purposes (for example, logs of data or specimens being transferred), and laboratory notebooks.
	Hard copies of data collection materials include an ID code and <u>do not have personal identifiers</u> . However, a code linking the data to the subject's personal information is stored separately from the data collection sheets, and is locked in a secure cabinet or room with limited access by authorized individuals.
X	Data are not collected on paper. Most data will be collected on Netbook Computers programmed with the questionnaires.
	Other (describe):
Electronic Databases: Indicate your choice but typing an X in the appropriate box on the left : <i>Note: A de-identified version of the database should be used for data analysis except in instances in which identifying information is prerequisite for coding or analysis. Databases that retain identifying information require a higher degree of electronic security.</i>	
	The study is minimal risk and data collected are not sensitive in nature. <u>No personal identifiers</u> are included in the electronic database. Any electronic documents that link IDs to identifying information are stored on a computer in accordance with JHSPH Data Security guidance.
X	<u>Personal identifiers</u> are included in the database. The data are stored on a computer that is password protected with a secure server. Transfer or storage on portable devices (e.g., laptops, flashdrives) is encrypted. The devices on which this information is stored are accessible only to individuals who need access to these data.

	<u>No personal</u> identifiers are included in the database but linkable identifiers exist separately and the data are sensitive in nature ( <i>e.g., substance use, mental health, genetic propensities, sexual practices or activities</i> ) such that disclosure could provide a risk to the individual. The codes are stored on a computer that is password protected with a secure server. Transfer or storage on portable devices ( <i>e.g., laptops, flash drives</i> ) is encrypted. The devices on which this information is stored are accessible only to individuals who need access to these data.
	Other (describe):

**c. Description of use of participants’ personal identifiers and plans for disposing of identifiers.**

Name and addresses will be collected to identify mothers for subsequent home visits. Data captured on all field computers will be encrypted with secure passwords and when data is transmitted to the Zvitambo/Harare office the transmission will be done over secure networks. Electronic databases will not include personal identifiers, but will include the study ID. A separate file linking the Study ID with the personal identifiers will be stored on main computer in the Zvitambo/ Harare office that is password protected with a secure server.

**d. Description of plans for destroying data**

We do not plan to destroy the electronic data. Any paper forms used will be locked in a secure cabinet or room with limited access by specified individuals and destroyed after it has been computerized or is no longer required and after at least 7 years of storage.

## **6. Recruitment Process**

Please see the recruitment process described under study procedures for the main trial.

## **7. Consent process and documentation**

**a. Countries where the research will take place and the languages into which each consent document will be translated**

The entire project will be conducted in Zimbabwe.

<b>Country</b>	<b>Consent Document (Indicate “All”, or specify each document when translations vary)</b>	<b>Languages</b>
Zimbabwe	<p><u>All</u></p> <ul style="list-style-type: none"> <li>• Trial Consent for Standard of Care Clusters</li> <li>• Trial Consent for Infant Feeding clusters</li> <li>• Trial Consent for WASH clusters</li> <li>• Trial Consent for Infant Feeding + WASH clusters</li> <li>• Consent for specimen archival and shipping</li> <li>• Consent for EE Sub-study- HIV+ mothers</li> <li>• Consent for EE Sub-study HIV- mothers</li> </ul>	Shona, Ndebele, and English

## **b. Who will obtain informed consent from participants, how, when and where**

The informed consent process will take a number of stages including a process of community engagement, followed by individual consent of eligible women.

### ***Community Engagement***

Three relevant groups will be engaged in order to obtain approval and assent to implement various program activities. These groups include (1) the Community Leadership, (2) Health Workers, and (3) Reproductive-aged women and their families.

(1) Community Leadership has been continuously informed about this study since planning for it began in about 2009. Investigators met with counselors (elected community representatives), traditional chiefs, district administrators, and the leadership of the Rural District Councils. Following their agreement, Project Agreements were signed between each district and Zvitambo. The project agreements detail the community based activities of the SHINE study and define the role of existing structures – the District Social Services Committee and the full council of the rural districts (the Rural District Council) in community engagement. In their roles as community representatives, members of the full council will aid towards communicating study objectives and strategies, communicating views and perceptions of the community, community engagement to maximize uptake/success and proactively dispel misconceptions in the community. Also, in their administrative roles within the Rural District Council, they will ensure the coordination of existing and new community development programs to ensure that they do not compromise the study. Zvitambo staff have also planned and implemented the aforementioned health systems strengthening activities with district provincial and national health authorities. The Provincial Medical Director has provided assent to randomize clusters in these districts for the trial and deliver randomized program activities through the health delivery system.

(2) Health Workers: Prior to beginning activities for this trial, information sessions will be held with health workers in the districts. These sessions will provide information about the trial and discuss the roles and responsibilities of research staff and linkages with the health delivery system.

(3) Reproductive-aged women and their families: Brochures describing the study will be distributed by Village Health Workers to women of reproductive age during pregnancy surveillance, available in antenatal and well-child clinics, and available in other public places to inform the community (**SHINE Community Brochure\_v5, SHINE EED Substudy Community Brochure v1, SHINE Early Child Development Assessment Community Brochure v1 and SHINE Community Brochure\_ Post Recruitment v1**).

### **Individual Consent Process**

The Village Health Worker will conduct routine surveillance for pregnancy using a system designed to detect pregnancies as early as possible in this setting. This is part of the health system strengthening process instituted prior to this trial. Once the VHW identifies a woman who is pregnant, he will ask her if a DC can visit to describe the project. If she assents the Village Health Worker will refer the mother to the DC using only the Village Health Worker ID, the mother's line number and the date of her LMP in that Village Health Worker's register – thus, the referral will be done without any personal identifiers. The Village Health Worker will accompany the DC within two weeks to the mother's home and introduce them. The DC will describe the study and elicit her consent for participation using the treatment group specific consent form that corresponds to the cluster in which she resides.

In cases where the mother dies but the infant is still alive, the new guardian will be asked for consent so that the infant can remain in the trial. We define guardian as the recognized

decision-maker for that baby; this person can be identified by the head of household and/or community leaders. Re-consenting will be undertaken using SHINE Form 28v2 Guardian SOC Consent Form, SHINE Form 29v2 Guardian IYCF Consent Form, SHINE Form 30 v2 Guardian WASH Consent Form, SHINE Form 31v2 Guardian WASH+IYCF Consent Form, SHINE Form 32v2 Guardian EED Sub-Study Consent Form

### **c. Vulnerable population enrolled?**

Yes, pregnant women are the target group for enrollment. All are from rural Zimbabwe, a setting where poverty is highly prevalent and resources are constrained. It is in this type of setting where the interventions being tested may have the most effectiveness.

We will translate all study forms from English into Shona and Ndebele to ensure accurate understanding of the trial and the processes in place. Illiterate women can provide consent using a thumb print in the presence of a witness.

Because young women in Zimbabwe are often culturally required to discuss decisions (like participating in a trial) with their husband and other family members, we will hold many meetings with community leadership, make brochures available to the general population, and include other family members in the consent discussion if the woman wants this.

We will emphasize participation is voluntary and they are free to refuse participation in any part of the study (i.e., refuse to answer any question posed or to participate in any process requested) or quit the study all together at any time during the study.

### **d. Waiver of consent requested?**

No.

## **8. Risks**

### **a. Description of risks**

Taking blood may cause discomfort, bleeding or bruising where the needle enters the body. In rare cases, it may cause fainting. There is a small risk of infection.

### **b. Frequency and severity of risks**

Rare risk of fainting and infection.

### **c. Description of measures to minimize risks**

All Zvitambo research staff have been trained on the protection of human participants and Good Clinical Practice and are experienced in speaking to people in a sensitive manner and addressing any issues that arise in a positive and supportive manner.

### **d. Description of level of research burden**

For each mother, we expect to enroll her by 24 weeks gestation and follow her until her baby is 18 months old. Thus each mother will participate for up to 25 months. The entire study (from enrollment of the first pregnant mother to the 18 month visit of the last enrolled mother) will run an estimated 3 calendar years.

For a women-infant dyad who participates the entire way through the study, she/they will be visited a total of 8-10 times over this 25 month period related to research data collection with an additional 3 brief visits to collect morbidity diaries and encourage their use for dyads in the EED Sub-study. The data collection visits will occur once or twice during early pregnancy to seek consent for the trial, twice more during pregnancy and 5 times post-partum (at 1, 3, 6, 12 and 18 months) for a total of 8-10 visits plus an additional 3 visits for morbidity diaries among EED Sub-study dyads. The baseline visit and any other visit in which the lactulose:mannitol test is conducted on the mother or infant will take at least 2 hours due the length of time urine must be collected. Other data collection visits that do not require this test will typically take 1 hour. The morbidity diary visit will take 5-10 minutes.

**Village Health Workers:** The number of visits to mothers and infants by village health workers for women and infants enrolled in the trial will be the same as specified by the MOHCC for Village Health Worker care of pregnant women and young children: each mother will be seen at least twice during pregnancy and at least monthly between delivery and 18 months post partum. Village Health Workers will also be contacted up to 10 times for research data collection during the course of the study – over a 5-year period. On 5 of these visits questionnaires will be administered and each questionnaire takes approximately 45 minutes. Village Health Workers will also undergo semi-structured observations (shadowing) for up to 4 times during their normal activities (e.g. home visits, group education, report writing, and travelling).

**e. Description of how participant privacy will be protected**

Privacy will be protected a number of ways for this trial. All paper forms with identifiable information will be stored in locked cabinets at field offices or study headquarters in Harare. The vast majority of data will be collected electronically via direct entry to netbook computers in the field. All data files on the netbooks will be encrypted as will all data transfers from the field to the data center in Harare. HIV tests results will be held in strictest confidence as per national policy in Zimbabwe. As much as possible, all forms or specimens will be labeled only with ID numbers and the linkage between this ID number and identifying information will be held in a secure location. Interviews conducted at the household will be done in a private location away from other adults or older children as appropriate. Maternal HIV status may be measured for all women on the baseline blood and at 32 weeks gestation, but these results will be used only during analysis after personal identifiers are stripped from the HIV result data to interpret primary outcomes by maternal HIV status. Thus, only if women have agreed to learn their status (by either agreeing to rapid testing by the DC at the baseline or 32 weeks gestation visit or having the test at the antenatal clinic which records the results on the antenatal card) will her result be made known to research staff. In addition, only women who agree to learn their status will be eligible for the EED Sub-study. We anticipate the number of women refusing to learn their status will be very low; current PMTCT program data indicates that 99% of pregnant women in the study districts know their HIV status at delivery.

In a community-based trial, people can be stigmatized even if the data and analysis are stripped of all personal identifiers if investigators name the group in reporting a sensitive finding. Recognizing risk of group harm from incidental findings, the steering committee will ensure that publications and public pronouncements of future study findings containing group associations are done with caution, respect for the group under study, and precision.

## 9. Benefits

### a. Direct Benefits

There are a number of direct benefits to participants in this study. All participants will be from communities in which a systematic health system strengthening process has occurred over the past 2-3 years. In addition, all households will receive a Blair style ventilated improved pit latrine, one half during the mother's pregnancy, and one half at the end of the trial, or upgrades to an existing latrine to bring it to MOHCC standard. All subjects will be visited regularly by a study nurse who will use his/her professional judgment regarding referral of the woman or her infant for care. At all postnatal visits, mothers will receive a small non-monetary gift to thank them and their infant for their time in participating in data collection. The total value of gifts over the course of the study will not exceed \$10.

### b. Societal Benefits

There are significant potential societal benefits from this study. If the results demonstrate that it is critical to prevent EED from occurring in order to improve the growth of young children, this would provide significant evidence to support a much more aggressive combined sanitation-hygiene and infant nutrition approach in countries with similar settings as rural Zimbabwe.

## 10. Payment

There is no payment for participation.

## 11. Drug Products, Vitamins, Dietary Supplements and Devices

### a. Drug, Vitamin, and Dietary Supplement products

#### Nutributter infant diet supplement

#### *i. Rationale:*

We selected Nutributter (along with education to the mother) as the randomized nutrition intervention because it is designed to fill key nutrient gaps of breastfed infants in environments where infant malnutrition is common (<http://www.nutriset.fr/en/product-range/produit-par-produit/nutributter.html>). Our formative research showed that Nutributter closed all the key nutrient gaps of rural Zimbabwean breastfed infants (Paul et al. 2010) The dose of 20 g daily is delivered in a single sachet of a lipid-based fortified paste mixed into at least 2 complementary food servings.

#### *ii. Dose Decision*

After the age of 6 months, breast milk alone is no longer sufficient to provide all essential nutrients to infants. In contexts like rural Zimbabwe, infant foods are typically low in numerous essential nutrients, and Nutributter is a product specifically designed to provide the daily recommended allowance of all micronutrients (Dewey et al. 2009).

**Table 12: Target nutrient composition of LNS for children (per daily ration)**

<b>Nutrient</b>	<b>Nutributter -LNS-20gM</b>
Ration (g/day)	20
Total energy (kcal)	<b>118</b>
Protein (g)	2.6
Fat (g)	<b>9.6</b>
Linoleic acid (g)	<b>4.46</b>
$\alpha$ -Linolenic acid (g)	<b>0.58</b>
Vitamin A ( $\mu$ g RE)	400
Vitamin C (mg)	30
Vitamin B <sub>1</sub> (mg)	0.3
Vitamin B <sub>2</sub> (mg)	0.4
Niacin (mg)	4
Folic acid ( $\mu$ g)	80
Pantothenic acid (mg)	1.8
Vitamin B <sub>6</sub> (mg)	0.3
Vitamin B <sub>12</sub> ( $\mu$ g)	0.5
Vitamin D (IU)	200
Vitamin E (mg)	6
Vitamin K ( $\mu$ g)	30
Iron (mg) <sup>1</sup>	<b>6</b>
Zinc (mg)	<b>8</b>
Cu (mg)	<b>0.34</b>
Calcium (mg)	<b>280</b>
Phosphorus (mg)	<b>190</b>
Potassium (mg)	<b>200</b>
Magnesium (mg)	<b>40</b>
Selenium ( $\mu$ g)	<b>20</b>
Iodine ( $\mu$ g)	90
Manganese (mg)	<b>1.2</b>

<sup>1</sup>Target iron content has been reduced from 9 mg in the current formulation of Nutributter to 6 mg, to reduce any potential risk associated with iron intake in malarial areas. As the daily ration will be divided into at least 2 meals during the day, the amount of iron consumed at any single meal should not exceed 3 mg. This falls within the amount of iron that a fortified processed

### **iii. *International Approvals***

Nutributter is not a regulated drug product in Zimbabwe. Plumpy Nut, which has the same base as Nutributter but provides micronutrient requirements in a 500 kcal dose, is used throughout the country for community-based treatment of severe acute malnutrition.

#### **FDA Regulated Drug Products**

(including dietary supplements being used to test for a drug effect):  
Nutributter is not a FDA-regulated drug product.

### **iv. *Justification and Safety Information***

Nutributter formulation is based on current knowledge of infant nutrient requirements. Our formative research showed that provision of Nutributter prevented low nutrient intakes by rural Zimbabwean infants, but nutrient intakes did not exceed the Upper Limits set by the Institute of Medicine (Paul et al., 2009).

### **v. *Responsibility for Drug Management***

Nutributter sachets will be managed by the Intervention Nurses, who will distribute sachets to the Village Health Workers randomly allocated to deliver the nutrition intervention. Provision of the Nutributter sachets to VHWs and to mothers will be monitored by the trial as we monitor intervention fidelity and adherence.

## **b. Medical Devices**

**HemoCue Hb 301 machine** (HemoCue AB, Angelholm, Sweden): Hemoglobin is measured on 10uL of venous blood, as previously described (van Kempen E J and Zijlstra W G. Spectrophotometry of hemoglobin and hemoglobin derivatives. *Advances in Clinical Chemistry*, vol 23, 199-257 (1983)). The Hb301 haemoglobinometer has been factory calibrated against the ICSH (International Council for Standardization in Hematology) method and shown to have excellent correlation with the gold standard. The precision of the machine is excellent, with a CV  $\leq 1.1\%$ ; the accuracy of the machine is within 7%, in conformity with CLIA'88 regulations (Federal register: Clinical laboratory improvement amendments of 1988; Final rules and notices. 28 February 1992, 7188-7288). In independent evaluations, it was found to have excellent linearity over a wide range (4-18 g/dL), a low level of imprecision (CV 0.4-0.7%) and an overall performance limit within 6% for 96% of samples, making it extremely reliable for clinical use (Morris et al, 2007).

**ADC Advantage 6021 Automatic Electronic Blood Pressure Monitor** (American Diagnostic Corporation, Hauppauge, NY, USA). This device is manufactured to meet the European and United States standards for non-invasive blood pressure monitors: EN-1060-1/1995, EN1060-3/1997, EN1060-4/2004 and AAMI/ANSI SP10. Clinical performance tests were carried out in the UK using the British Hypertension Society clinical protocol to measure the accuracy of this product, which was graded 'AA' for systolic/diastolic accuracy



by independent investigators, the highest grading available for BP monitors. The measuring range (systolic/diastolic) is 30-280mmHg, measuring resolution is 1mmHg and accuracy is within  $\pm 3$ mmHg.

**BodyStat 1500 MDD** (BodyStat, Isle of Man, British Isles) is a non-invasive, portable, bioelectrical impedance analyzer which can measure reactance, resistance and phase angle to enable calculation of lean and fat mass. It works by passing a safe battery-generated signal (i.e. an undetectable electric current) through the body and measuring the impedance at two frequencies (5kHz and 50kHz) using two clips attached to the body.

**Knemometer**, developed in collaboration with the Danish Institute of Biomedical Engineering, resembles a pair of electronic calipers and records the knee-heel length to a resolution of 0.01mm (Michaelsen *et al.*, 1991).

## 12. Safety monitoring

### a. Participant safety monitoring

Participants will be visited on a regular basis in their homes by a licensed research nurse (DC). This staff member will use his/her best professional judgment regarding when to refer the mother or her infant for care. We do not anticipate any safety concerns with the interventions being tested. If an unusual situation occurs the nurse will report it to his/her supervisor and the health center for resolution, and will complete an SAE form if appropriate.

### b. Data Safety Monitoring Board (DSMB)

Since this is a randomized trial, we feel it is appropriate to have a DSMB.

#### i. *DSMB membership, affiliation and expertise*

The SHINE trial DSMB will be formed of:

**Professor Simon Cousens** (Professor of Statistics, London School of Hygiene and Tropical Medicine) will chair the committee.

**Dr Tariro Makadzange** (Lecturer, Department of Internal Medicine, University of Zimbabwe)

**Dr. Hilda Mujuru** (Sr. Lecturer, Department of Paediatrics, University of Zimbabwe).

The DSMB will meet up to three times, the final schedule will be determined in consultation with them at the first meeting.

#### ii. *The charge to the DSMB*

We will draft a charge to the DSMB just prior to the first meeting and finalize it in consultation with the DSMB members. We consider the reporting responsibility be to the investigative team and not the sponsors of the trial.

#### iii. *Plans for providing DSMB reports to the IRB*

All DSMB reports will be sent to the investigative team who will review it and respond to any concerns expressed in the report. Both the report and the investigative team's response will then be forwarded to all the IBRs involved.

**c. Plans for interim analysis and stopping rules**

Current plans call for, at most, one interim analysis when approximately one-half the mother-infant dyads have completed 18 months post-partum follow-up. However, this will be discussed with the DSMB at the first meeting to determine if they think fewer or more interim analyses are appropriate. By the time this halfway point is reached, almost all recruitment will have been completed. As a result we do not anticipate using any formal statistical stopping rules for this trial.

**d. Monitoring of trial activities**

The SHINE Monitoring Plan details all planned and systematic actions that have been established to ensure the SHINE trial is performed and the data are generated, documented, and reported in compliance with the principles of Good Clinical Practice (GCP) and applicable regulatory requirements.

**13. Plan for reporting unanticipated problems and adverse events.**

The interventions involved in this trial including the universal strengthening of the MOHCC Village Health Worker program, the WASH interventions, and the IYCF interventions are all well accepted inputs for public health programs. We will be more aggressive about their implementation that has been done in the past, but no new separate interventions are being tested in this trial. Therefore, we do not anticipate any major adverse events that could be attributed to this study.

To minimize risk of peanut allergic reaction, Village Health Workers will be trained to test feed 1 teaspoon of Nutributter to the infant to ascertain any reaction. We do not expect to identify any cases of peanut allergy in the study population. Peanuts are a staple food in Zimbabwe yet peanut allergy is unknown. Even in the United States – where there is much attention given to peanut allergy – severe allergies to peanut are uncommon among infants under two years of age. Both the overall prevalence of allergy and the prevalence of severe allergy are very similar to those for milk (1.4-2.0% for any allergy and 0.6-0.7% for severe allergy) (Gupta *et al*, 2011). There is very little information available on peanut allergies in developing countries, but prevalence may be lower than in the U.S. (Yang *et al*, 2010). In an on-going trial giving this product to several thousand infants in Burkina Faso and Malawi, not a single reaction has been observed (iLiNS/ U California, Davis, personal communication). In addition, both the American Academy of Pediatrics and the National Institute of Allergy and Infectious Diseases advise no general restriction on introduction of potentially allergenic foods in infancy (after 6 months of age, when complementary feeding is recommended to commence) (Greer *et al*, 2008; Burks *et al*, 2011).

**Adverse Event and Serious Adverse Event reporting**

An Adverse Event (AE) is defined as any untoward medical occurrence in an enrolled study participant, regardless of its relationship to trial activities.

Serious Adverse Events (SAEs) are defined by the ICH Harmonised Tripartite Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting (1994), i.e. an incident that involved an enrolled study participant and includes one or more of the following:

1. Results in death of a study participant

2. Is life threatening to a study participant (ie at risk of death at the time of the event)
3. Requires unplanned hospitalization, OR prolongation of existing hospitalization of a study participant
4. Results in persistent or significant disability/incapacity of a study participant
5. Is a congenital anomaly or birth defect of a study baby
6. Is any other important medical event considered serious by the investigator of a study participant (eg anaphylaxis requiring emergency treatment but not resulting in hospitalization). An important medical event should carry a real, not hypothetical, risk of one of the above events (1-5) if prompt medical action were not taken.

Where the study team first learns about an AE or SAE outside of a scheduled study visit (e.g. report to the field hub by a VHW, or other staff member), a home visit will be made as soon as possible to collect information about the event. Where an AE or SAE is reported at a scheduled study visit, information will be collected during the visit. AEs and SAEs will be reported on an Event Form. The staff member reporting the event will complete part 1 of the Event Form and submit it to the Research Nurse Supervisor or Nurse Intervention Manager at the Hub the same day. The RNS/NIM will review all Event Forms within 24 hours of the form being completed, and will fill in Part 2 of the form. The RNS/NIM will pass the form to the Field Data Supervisor in the hub, who will create an electronic record of the event on the database, within 24 hours of receiving the form; this will generate an Event number.

The Compliance Team at Zvitambo will review all Event forms within 48 hours of receipt. The compliance team will adjudicate the event and check Part 1 and Part 2 of the form, then provide a summary of the event(s) and decisions made. Adverse Events and SAEs will be judged as either unlikely, uncertainly, probably or definitely related to the study interventions or procedures.

Since this is a community-based trial, we anticipate a large number of AEs and SAEs that are unrelated to trial activities. Because of the rural location and limited means of communication within the study districts, it is unlikely that the trial team will find out about most AEs and SAEs in real time.

### **Reporting of events to the local IRB (MRCZ) and international IRB (JHU)**

Events will be reported to MRCZ according to the following guidelines:

#### **1. AEs and SAEs *related* to trial activities:**

Where AEs and SAEs are judged to be uncertainly, probably or definitely related to the study interventions or procedures, they will be reported to MRCZ and JHU IRB under the expedited reporting time-frame (MRCZ: 3 days for SAE, 7 days for AE; JHU: within 10 days of the investigators learning of the event).

The potential adverse events that may be related to our interventions (although have not been described to date by the manufacturers or distributors) are reactions to Nutributter and accidental ingestion of Waterguard. To be certain that we detect any reactions to Nutributter, we will ask a series of questions during home visits, from 6 months of age, to all mothers whose babies are randomized to receive Nutributter. Any adverse events will be followed up with a full AE/SAE narrative in the field, and expedited reporting to MRCZ, JHU IRB and the DSMB. Any cases of accidental Water Guard ingestion will similarly undergo expedited reporting to MRCZ, JHU IRB and the DSMB.

#### **2. AEs and SAEs *unrelated* to trial activities:**

AEs and SAEs that are judged unlikely related to study interventions and procedures will be reported annually to MRCZ and JHU IRB in a table (split by type of AE or SAE). Clinical events that led to attendance at a clinic will be reported, to capture those events that are likely to be grade 3 or 4 (serious or life threatening). Sufficient data will not be captured to enable grading of every AE symptom according to DAIDS tables.

**3. Reporting of abnormal clinical investigations conducted as part of study protocol:**

Abnormal results which are grade 3 or 4 adverse events according to DAIDS tables (or qualify for referral according to Zimbabwean guidelines, where not covered by DAIDS tables), will be reported to MRCZ and JHU IRB in an annual table. These will be graded as shown in Tables 14 and 15:

**Table 13: Classification Table for Women enrolled in SHINE**

<b>Parameter</b>	<b>Grade 3 Severe*</b>	<b>Grade 4 Potentially life threatening*</b>
Haemoglobin (known HIV positive woman)	6.5 – 7.4 g/dL	<6.5 g/dL
Haemoglobin (known HIV negative woman, or HIV status unknown)	7.0 – 8.9 g/dL	<7.0 g/dL
Hypertension	≥180 mmHg systolic OR ≥110 mmHg diastolic	Life-threatening consequences (eg malignant hypertension) or hospitalization indicated (other than clinic visit)
Hypotension	Symptomatic, requiring admission for IV fluids	Shock requiring vasopressors or mechanical assistance to maintain blood pressure
Malnutrition	MUAC <230 mm	MUAC <190 mm OR bilateral pitting oedema
Proteinuria	4+ on urinary dipstick testing	NA

Hematuria	Gross hematuria	Hematuria requiring transfusion
Maternal depression	Alteration in personality-behaviour causing inability to perform usual social and functional activities	Behaviour potentially harmful to self or others (eg suicidal and homicidal ideation or attempt, acute psychosis) OR causing inability to perform basic self-care functions.
New HIV infection	NA	NA
New urinary schistosomiasis	NA	NA

\*Grading based on Division of AIDS (DAIDS) table for the grading of adverse events (published December 2004, with clarification August 2009) where available. Malnutrition parameters based on Zimbabwe MOHCC criteria for referral for nutritional rehabilitation, taken from 'Malnutrition Management of Acute Malnutrition in Zimbabwe' (Version 1; June 2011).

**Table 14: Classification Table For Infants Enrolled In SHINE**

<b>Parameter</b>	<b>Grade 3 Severe*</b>	<b>Grade 4 Potentially life threatening*</b>
Haemoglobin (infant aged 22-35 days)	7.0 – 7.9 g/dL	<7.0 g/dL
Haemoglobin (infant aged 36-56 days)	6.0 – 6.9 g/dL	<6.0 g/dL
Haemoglobin (infant or child aged ≥57 days)	7.0 – 8.9 g/dL	<7.0 g/dL
Malnutrition in a child <6 months	Weight-for-length Z-score <-3 OR MUAC <110 mm	Grade 3 criteria PLUS any IMCI danger sign

	Bilateral pedal oedema OR  Infant too weak to feed or suckle effectively	
Malnutrition in a child $\geq 6$ months	Weight-for-height Z-score <-2 OR  Bilateral pedal oedema OR  MUAC <125mm	Grade 3 criteria PLUS any IMCI danger sign
New HIV infection	NA	NA

\*Grading based on Division of AIDS (DAIDS) table for the grading of adverse events (published December 2004, with clarification August 2009) where available. Malnutrition parameters adapted from Zimbabwe MOHCC criteria for referral for nutritional rehabilitation (Malnutrition Management of Acute Malnutrition in Zimbabwe, Version 1; June 2011) and from published data on predictive value of MUAC in infants <6 mo old (Mwangome 2012).

If any abnormal investigation also fulfils SAE criteria (eg severe anemia leading to hospitalization), it will be reported in the SAE section of the Tables.

If any mother or child enrolled in the trial is found to be acutely unwell at a scheduled home visit, they will be assessed by the study research nurse as per their training. If a mother or child needs to be referred to clinic for assessment then an AE form will be completed.

#### **Additional reporting to the international and local IRB**

We will also report the following to the JHU IRB and MRCZ within 10 working days of learning of the event: i) information that indicates a change to the risk:benefit ratio of the research; ii) withdrawal from marketing of a product being used (Nuributter or Waterguard); iii) changes to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a research participant; iv) incarceration of a participant; v) any event that requires prompt reporting to the sponsor; vi) complaint of a participant where the complaint indicates unexpected risks or the complaint cannot be resolved by the research team; vii) protocol violations (meaning an accidental or unintentional change to the IRB approved protocol) or breaches that placed one or more participants at increased risk, or has the potential to occur again.

#### **Reporting of events to the DSMB**

Any grade 3 or 4 AEs or SAEs that are related to Nutributter or Water Guard will be reported to the DSMB within 3 days of the investigators learning of the event. All other SAEs (excluding abnormal laboratory results) will be reported to the DSMB every quarter, split by trial arm.

## 14. Other IRBs/Ethics Review Boards

In addition to the JHBSPH IRB, the following IRB will need to review and approve the project:

Medical Research Council of Zimbabwe, FWA# 00002409

Cornell University, FWA# 00004513 has entered into an authorization agreement with the JHBSPH IRB, agreeing they both will rely on the JHBSPH IRB for review and continuing oversight of the protocol. The University of London recognizes the MRC-Zimbabwe as having jurisdiction over research in Zimbabwe.

## 15. Supervision Roles and Responsibilities

**Zvitambo Prof. Jean Humphrey**, Professor at Johns Hopkins University and Director of Zvitambo is co-Principal Investigator and will have overall responsibility for the study. Within Zvitambo, teams led by the following co-investigators will have specific roles and responsibilities: **Naume Viola Tavengwa** (Assistant Director, Field Operations) will be responsible for overseeing the implementation of study interventions and field operations for effective module delivery by Village Health Workers in line with government policy and programming guidance and for communication between organisations and key government/community level stakeholders. She will lead a team that oversees improved research visit outcomes. **Robert Ntozini** (Associate Director IT/Data Management/Statistics) will set up the field and laboratory information systems and oversee data management processes and statistical analyses; **Batsirai Mutasa** (Field Data Coordinator) will coordinate and supervise field data management processes implemented by Field Data Supervisors at each hub; **Dr Andrew Prendergast** (Clinician Scientist from University of London, resident in Zimbabwe) will be responsible for developing the human and technical capacity of the Zvitambo Laboratory to receive, process and analyze samples and will oversee the implementation of clinical assessment protocols and of field research protocols by Research Nurses. **Florence Majo** (Research Nurse Coordinator) will coordinate and supervise all field research activities by Research Nurses and Data Collectors; **Kuda Mutasa** (Laboratory Manager) will manage the field laboratory Technicians, field laboratories and central laboratories in line with government policy guidance and oversee the implementation of all specimen processing assays;; **Virginia Sauramba** (Research Administration and Compliance Manager) will manage, monitor and oversee compliance activities to ensure Good Clinical Practice procedures are followed and implemented in line with regulatory ethical guidelines and government policy guidance and also ensuring linkages with relevant Johns Hopkins University and SPH offices and department.

## 16. External Investigator Roles and Responsibilities

Dr Mduduzi Mbuya, who was Associate Director, Implementation Science was responsible for the design of randomized interventions, the development of implementation research protocols and oversaw the delivery of study interventions now working for GAIN, USA, will continue to contribute towards SHINE manuscript writing and lead some of the manuscripts.

*Ministry of Health and Child Care:*

The Ministry of Health and Child Care co-investigators include **Goldberg Mangwadu**, Director of Environmental Health who is also co- Principal Investigator of the study; **Cynthia Chasokela**, Director of Nursing Services; and **Ancikaria Chigumira**, Deputy Director Nutrition. The study team works closely with the Provincial and District Health Executives towards the planning and implementation of health systems activities. These authorities ensure the relevance as well as the consistency of these activities with prevailing policy and programming guidance

*Collaborating Universities:*

A multidisciplinary team of researchers within and outside Zimbabwe will support the Zvitambo and Ministry of Health and Child Care team at all stages of the project's design and implementation with expertise in nutrition, epidemiology, statistics, program implementation and public health program evaluation. Co-investigators **Dr. Larry Moulton**, **Dr James Tielsch**, and student investigator **Tatenda Mupfudze** (PhD) from Johns Hopkins School of Public Health. And co-investigators **Dr Rebecca Stoltzfus**, **Dr. Andrew Jones** and student investigators **Rukundo Kambarami** (PhD), **Cynthia Matare** (PhD), **Laura Smith** (PhD) and Dadirai Fundira (PhD) from Cornell University, and **Dr. James Church** (PhD) and **Dr Joseph Piper** (PhD) from University of London will visit the project on a regular basis to provide planning and implementation support. **Jaya Chandna** (ECD specialist from the University of Liverpool) will oversee piloting, training and ongoing conduct of the ECD assessments.

## 17. Oversight plan for studies conducted at non-JHSPH sites

The JHBSPH PI and co-investigators are part of a study team who will manage the project along with colleagues from the Zimbabwe Ministry of Health and Child Care, Cornell University, and University of London. The project supervisory staff are experienced co-investigators and field managers. Quality control procedures are emphasized during project-specific training and this is reviewed on a regular basis by study co-investigators.

Our staff in Zimbabwe have received training in human subjects research from the Medical Research Council of Zimbabwe. Newly hired research staff for this project will undergo human subjects training in addition to project-specific task training. This will involve presentations and discussions on the importance of voluntary participation, informed consent, confidentiality, data security, and sensitivity to the participants in the research. These presentations will be reinforced with role playing and direct supervision of the process during the early stages of the study.

**Table 15: Roles and Responsibilities Matrix for IRB Application. For the following, indicate “P” for “Primary”, “S” for “Secondary” as appropriate to role and level of responsibility.) Add additional items if useful.**



	JHSPH	Zvitambo	Cornell Univ.	Univ. of London	Zimbabwe Ministry of Health and Child Care
Primary Grant Recipient	Primary scientific and administrative responsibilities				
Subcontractor		Implementation of the trial	Provision of nutritional expertise	Provision of immunologic scientific expertise	Coordination of project activities with MOHCC system

1	Human subjects research ethics training for data collectors	S	P	S	S	S
2	Day to day management and supervision of data collection	S	P	S	S	S
3	Reporting unanticipated problems to the JHSPH IRB/Sponsor	S	P			
4	Hiring/supervising people obtaining informed consent and/or collecting data	S	P	S	S	S
5	Execution of plan for data security/protection of participant data confidentiality, as described in Sect. 5.	S	P			
6	Biospecimen processing, storage, management, access, and/or future use	S	P	S	S	

## 18. Creation of a biospecimen repository

### a. Source and purpose of the biospecimens

Aliquots of plasma, serum, PBMCs, feces, urine and saliva from each collection time point will be cryopreserved at -80°C for all women following informed consent. Aliquots of plasma, serum, cell pellets and feces from all infants at 18 mo of age will be cryopreserved at -80°C following informed consent. Aliquots of plasma, PBMCs, RNA (see below), feces, urine and saliva, collected from all infants in the EED substudy at 1, 3, 6, 12 and 18 mo of age, will be cryopreserved at -80°C following informed consent. Samples will be used to measure pre-specified analytes and will be archived for subsequent analyses of new biomarkers that will inform the aims of the trial, or future studies of maternal/child health that can be usefully addressed with this specimen archive. Specific consent will be obtained to extract DNA from stored biospecimens of women and infants using **SHINE\_Form9v5\_Specimen Storage and Shipment Consent Form** (see below).

### b. Biospecimen storage

All samples will be stored in the Zvitambo laboratory, Harare, Zimbabwe in a bank of -80C freezers that are kept in a locked store room with immediate back-up generator in case of power failure. Samples will be stored for up to 25 years from the date of collection. We will add an archive flag to the participant IDNO to specify how specimens are to be processed. Eg we will have IDNO=1023-0 or IDNO=1023-1 for archive and don't archive samples. The full IDNO will be generated after the consent process when we have an indication of the method of processing based on the two options we provide to the participants during the consent process: 1) allow to store, and 2) do not allow to store. Barcode labels will incorporate the flag and the LIS can be programmed to prompt if any flagged samples are being used in assays.

### c. Sharing of biospecimens

Access to biospecimens by other researchers will be considered following review of a proposal by SHINE investigators and subject to approval by the Medical Research Council of Zimbabwe and/or Research Council of Zimbabwe, as appropriate. In particular, if any samples requiring export for analysis, approval will have to be granted by RCZ.

### d. Future specimen derivation and processing (cell lines, DNA/RNA, etc.)

Consent will be obtained to extract and store DNA from enrolled mothers and infants using **SHINE\_Form9v5\_Specimen Storage and Shipment Consent Form** and to extract and store RNA from infants enrolled to the EED substudy using **SHINE\_Form10v5Specimen Storage and Shipment Consent Form – EED Infants**. For RNA, blood will be stored in PAXgene RNA Tubes at -80C from infants in the EED substudy at 5 time-points (1, 3, 6, 12 and 18 mo of age) to characterize gene expression profiles in stunted versus non-stunted infants; this will enable us to explore mechanistic pathways to stunting using a non-biased approach. For DNA, cell pellets from infants at the 18 mo blood draw will be stored at -80C and DNA subsequently extracted. DNA will be stored for use in future studies of genetic determinants of infant growth and health. As stated in **SHINE\_Form9v5** and **SHINE\_Form10v5**, maternal and infant samples for women who do not give consent for long-term storage and shipment (including extraction and storage of DNA and RNA) will be specifically identified by bar-code and not used for future studies.

**e. Specimen coding and identification**

Samples will be bar-coded with no patient identifiers, and logged on an in-house Laboratory Information Management System that is only accessible on password-protected computers stored in locked offices.

**f. Certificate of Confidentiality protections**

Not applicable.

**g. Ability of participant to withdraw consent**

Participants will be required to sign a consent form to agree to storage and possible shipment of samples. The consent form states that participants are free to withdraw their consent at any time.

**h. Data and/or specimen use agreements**

The final dataset will include maternal-reported demographic, behavioural, morbidity data, observed and maternal-reported household data, and laboratory data from urine, fecal, and blood samples collected from the infant. The dataset will be stripped of all personal identifiers prior to release for sharing. It is an expectation of the Medical Research Council of Zimbabwe (MRCZ), one of the IRB's of record for the trial, that a Zimbabwean be a co-author on publications resulting from data collected on Zimbabwean human subjects. We will make publicly available through a website a description of the database and a description of the specimen archive that has been collected. Anyone who wants to conduct further data analyses should present a proposal of the intended use to a steering committee who will determine if the proposed study will generate important information and whether the trial data is relevant for the analysis. If so, the committee would assign at least one member of the trial team (including a Zimbabwean) to join this secondary data analysis team. This person would be responsible for obtaining ethical permission for the additional analyses from the committees that governed the trial - the MRCZ and the IRB of JHU, and for timely communication of findings with the MRCZ and any other relevant Zimbabwean authorities.

As noted in section 8e, in a community-based trial, people can be stigmatized even if the data and analysis are stripped of all personal identifiers if investigators name the group in reporting a sensitive finding. Recognizing risk of group harm from incidental findings, the steering committee will ensure that publications and public pronouncements of future study findings containing group associations are done with caution, respect for the group under study, and precision.

**19. Data Coordinating Center**

Not applicable.

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## **APPENDIX 3**

### **SHINE STATISTICAL ANALYSIS PLAN**

## STATISTICAL ANALYSIS PLAN

Version 2.0

July 23 2017

*Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial*

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## 1. INTRODUCTION

This document contains the statistical analysis plan for the SHINE study. The goal is to avoid data-driven analyses during and at the end of the study to the extent possible. Much of the content of this SAP is taken directly from the SHINE Study Protocol.

## 2. BACKGROUND

### 2.1. Study Overview

SHINE is a proof-of-concept, 2x2 factorial, cluster-randomized, community based trial in two rural districts of Zimbabwe that will test the independent and combined effects of protecting babies from fecal ingestion (Factor 1, operationalized through a WASH intervention) and optimizing nutritional adequacy of infant diet [Factor 2, operationalized through an infant and young child feeding (IYCF) intervention] on length-for-age Z score and haemoglobin concentration at 18 months of age. Pregnant women will be enrolled and they and their infants followed until the children are 18 months of age. Recruitment began in November 2012, with the aim of enrolling all women who become pregnant and are residing in rural areas of the Districts of Chirumanzu and Shurugwi at 8-14 weeks of gestation, for about two years of recruitment, to achieve an enrollment total of 4800 women. Recruitment ended in March 2015. Over the recruitment period, the cut-off of gestational age for recruitment eligibility was gradually liberalized to maximize recruitment. Due to delays in delivering the latrines to women enrolled in the WASH arms of the trial, the total sample size was increased by 10% to allow for sensitivity analyses including only women who received the intervention as planned (ie, before the birth of the infant); accordingly, a total of 5280 women were enrolled in the trial.

Within SHINE we will measure two causal pathways. The **program impact pathway** comprises the series of processes and behaviors linking implementation of the interventions with the two child health outcomes; it will be modelled using measures of fidelity of intervention delivery and household uptake of promoted behaviors and practices. We will also measure a range of household and individual characteristics, social interactions, and maternal capabilities for childcare, which we hypothesize, will explain heterogeneity along these pathways. The **biomedical pathway** comprises the infant biologic responses to the WASH and IYCF interventions that ultimately result in attained stature and hemoglobin concentration at 18 months of age; it will be elucidated by measuring biomarkers of intestinal structure and function (inflammation, regeneration, absorption and permeability); microbial translocation; systemic inflammation; and hormonal determinants of growth and anemia among a subgroup of infants enrolled in an EED substudy.

The Early Child Development (ECD) substudy will investigate the independent and combined effects of protecting babies from fecal ingestion (Factor 1, operationalized through a WASH intervention) and optimizing nutritional adequacy of infant diet [Factor 2, operationalized through an infant and young child feeding (IYCF) intervention] on early child development at 24 months as measured through assessment of gross motor, fine motor, language, cognitive and social development, together with disability and behavior measures, in a subgroup of children. Both the program impact pathway and the biomedical pathway will be taken into account (as above).

### 3. OBJECTIVES

The objectives of the trial have been published in full (SHINE Trial Team, Clin Infect Dis 2015; available at [https://academic.oup.com/cid/issue/61/suppl\\_7](https://academic.oup.com/cid/issue/61/suppl_7)).

### 4. RANDOMIZED INTERVENTION ARMS

The randomized interventions in the four cells of the trial are given in **Table 1**.

**Table 1: Randomized Arms**

<b><u>Standard Care: (“Latrine Later”)</u></b> <ul style="list-style-type: none"><li>• Exclusive breastfeeding promotion for all infants, birth to 6 months</li><li>• Strengthened PMTCT services</li><li>• Strengthened Village Health Worker system</li><li>• Latrine provided at end of study</li></ul>	<b><u>WASH: (“Latrine Now”)</u></b> <ul style="list-style-type: none"><li>• Standard care interventions</li><li>• Provide household ventilated pit latrine, water treatment solution, and monthly liquid soap.</li><li>• Provide interpersonal communication interventions promoting feces disposal in a latrine, HWWS, drinking water treatment, hygienic weaning food preparation, and preventing babies from putting dirt and animal feces in their mouths.</li></ul>
<b><u>IYCF: (“Latrine Later and Nutributter”)</u></b> <ul style="list-style-type: none"><li>• Standard care interventions</li><li>• Provide 20 g/d Nutributter from 6-18 months</li><li>• Provide interpersonal communication interventions promoting optimal use of locally available foods for complementary feeding after 6 months, continued breastfeeding and feeding during illness.</li></ul>	<b><u>Sanitation/Hygiene AND Nutrition: (“Latrine Now and Nutributter”)</u></b> <ul style="list-style-type: none"><li>• Standard care interventions</li><li>• All WASH interventions</li><li>• All IYCF interventions</li></ul>

### 5. RANDOMIZATION

The study area has been divided into 212 clusters, each equivalent to the catchment area of 1 to 3 Village Health Workers. Digital maps of administrative boundaries of the study area were obtained from the Central Statistics Office and using Google Earth, the positions of all homesteads and key landmarks (roads, clinics, schools, rivers) for the 2 districts were plotted. Large scale maps were created including all details and Zvitambo staff met with each of the 320 Village Health Workers and asked them to identify the borders of their catchment areas on the maps. (Additional VHWs were added during the course of the trial, bringing the total to 400). Data were also gathered from the Village Health Worker registers regarding the number of households and reproductive-aged women in their catchment, number of currently pregnant women, and number of <2 year old children. Cluster boundaries were finalized by grouping together Village Health Workers who work together or whose catchment areas include homesteads that are close to the catchment boundaries into the same cluster and putting VHWs that worked alone into one cluster while also attempting to achieve about the same number of households, pregnant women, and young children in each cluster.

Randomization was stratified on district, so that the same numbers of each of the four cluster types appeared in each district, within 1. Exact balance was not possible, because 111 clusters were formed in one district and 101 in the other, neither of which was a multiple of four. Forming appropriate clusters was deemed more important than achieving exact balance.

A highly constrained randomization scheme, allocating 53 clusters to each of the four trial cells, was implemented to achieve balance for key variables to within 10-25 percentage points with balance for each of the marginal intervention comparison arms (arms 1+2 vs. 3+4 and 1+3 vs. 2+4). The variables constrained upon were:

To within +/- 10%

- Median distance of households to the nearest health center
- Mean distance of households to the nearest health center
- Mean latitude of cluster centroids
- Mean longitude of cluster centroids
- Total number of women of childbearing age
- Settlement type: proportion 'traditional communal' as opposed to 'resettled'
- Proportion of households with 'best water access' (those households within a cluster that were located within a radius of 500m of a perennial water point, either borehole or well)
- Proportion of households with 'worst water access' (those households within a cluster that were located outside a radius of 1500m of a perennial water point, either borehole well)
- Proportion of households with any latrine
- Proportion of households with latrine <half full
- Proportion of households with latrine <half full, and with handwashing facilities

To within +/- 20%

- Standard deviation of latitude of cluster centroids (degrees)
- Standard deviation of longitude of cluster centroids (degrees)

To within +/- 25%

- Mean distance to nearest water source

Study staff, including the PI, Sponsors, and collaborators will in general not be producing or viewing data summaries by study arm of any of the outcome data (morbidity, mortality, anthropometry, laboratory analyses). Exceptions may be made for some reporting to the DSMB.

## **6. SCHEDULED DATA COLLECTION TIME POINTS**

### **6.1. Referral (target: 2-<14 weeks gestation)**

There are 400 Village Health Workers working in the two study districts. They will visit all women aged 15-49 years every 5 weeks and will enquire about a woman's last menstrual period (LMP) and offer urine dipstick pregnancy tests to mothers in the community who have missed a period. For women who have a negative pregnancy test followed by a positive pregnancy test, or who can provide a reliable LMP date, the VHW will also request the woman's assent to be visited by a SHINE Data Collector (DC) to learn about SHINE and be offered the opportunity to be enrolled in the study.

### **6.2. Enrollment/Informed Consent Visit (target: 8-<14 weeks gestation)**

#### **6.2.1. Inclusion/Exclusion criteria**

Inclusion: Women residing in randomized clusters within the study districts under monthly prospective pregnancy surveillance of a Village Health Worker, who have a negative pregnancy test followed by a positive pregnancy test, or who have a first positive pregnancy test with a known last menstrual period date. Initially, we restricted recruitment to women <14 weeks gestational age. This was liberalized three times to increase recruitment rates.

Exclusion:

- The goal was to recruit women between 10-14 weeks gestation, so at the beginning of the trial pregnant women more than 14 weeks gestation were excluded. However, this cut-off was liberalized 3 times over the life of the trial to maximize recruitment: to 18 weeks (August 22, 2013), 24 weeks (January 3, 2014), and < parturition (October 20, 2014).
- Women residing in the study districts who were already >14 weeks gestation (the cut-off in force at the beginning of the trial) when trial recruitment begins
- Women who are at the gestational age cut-off in force at the time of identifying their pregnancy
- Women residing in the study districts who become pregnant during the enrolment period but do not consent to join the trial
- Women who reside in urban areas of these two districts
- Women who are resident as employees at a homestead or who are living on rented premises

**6.3. Main Data Collection Visits.** Initially (November 20, 2012), the protocol specified target dates (in gestational age or infant age) only for each research visit. Amendment version 4 (implemented March 25, 2013) articulated that a research visit should take place within 2 weeks of the target date. Amendment version 7 (implemented May 15, 2014) extended the allowable range of each research visit until the next research visit opened (2 weeks before its target date) except for the 18 month; for the 18 month visit Version 7 specified 104 weeks (24 months) as the end of the allowable range for the visit. Version 13 (implemented August 3, 2015) extended the 18 month window to 130 weeks (30 months). In version 13 of the protocol (October 5 2015) a new visit was added to assess early child development at 24 months (104 weeks, allowable window 102-112 weeks).

## **7. SAMPLE SIZE AND POWER CONSIDERATIONS**

A total sample size of 4800 pregnant women was planned, 1200 in each of 4 treatment arms. Due to delays in delivering the latrines to women enrolled in the WASH arms of the trial, the total sample size was increased by 10% to allow for sensitivity analyses including only women who received the intervention as planned (i.e., before the birth of the infant); accordingly, a total of 5280 women were enrolled in the trial.

The primary outcomes are length-for-age Z-score and haemoglobin concentrations at 18 months of age, stratified by maternal HIV status. However, because of the widespread attention to the use of stunting as an indicator of population nutritional status, we wanted to make sure the study had sufficient power to find an important reduction in stunting.

The main trial inference will be based on outcomes of infants of HIV-negative women, of whom we expect at least 4080 (more likely, 4200) based on a 15% HIV-positive rate among pregnant women. Allowing for 20% loss of evaluable infants at 18 months (this includes pregnancy losses, infant mortality and loss to follow up), we expect about 816 measurements in each of the four study arms. With Type I error of 5%, and power of 90%, and control group stunting (height-for-age Z score <-2) of 30%, and the assumption that the effects of the two interventions will be additive, we will be able to detect a reduction of about 8 percentage points, i.e. from 30% to 22.0%, for either of the interventions (Hayes, 1999). Among the expected 720 pregnancies in HIV positive women, we expect a minimum of 25% loss through fetal loss, etc. resulting in 540 total children at 18 months of age, or

135 per treatment group. This is based on an assumed coefficient of variation of the true proportions of 0.43, and an effective loss of 33% of sample size due to cluster size variability, which corresponds to a design effect of 2.5. Note that the largest design effect reported by Katz for stunting in several countries was only 2.6 (Katz, 1995). Using the design effect of 2.5, we can detect a shift of 0.2 weight-for-age and length-for-age Z-scores, assuming a within-randomized-cluster variance of 1.25.

*Hemoglobin:* Assuming a SD at 18 months of 12.8 g/L, we will be able to detect a shift of 2.6 g/L.

*ECD Substudy Power Calculation:* The ECD substudy will be based on outcomes of infants of HIV negative women who have children who are turning 24 months from March 7, 2016 to April 30, 2017, of whom we expect at least 3000 women and their children to be eligible. Expecting at least 20% loss to follow up, 2600 women would be eligible with their infants at the age of 2 years.

Based on previous trials of early child development, an effect size of 0.3-0.4 shift in standard deviation on measures of ECD such as the Bayley's Scale of Child Development (equivalent to the MDAT) have been seen. With an ICC of 0.05-0.2 and 5% type I error with >80% power to detect effect size of 0.2-0.4 using the MDAT, a minimum of 260 and maximum of 1320 children would be needed, as shown in **Table 2**.

**Table 2 – Early child development substudy sample size calculations**

Run	Effect Size (SD shift)	ICC	#Evaluated /cluster	#Clusters/arm	Total Clusters*	Total Children	
A	0.2	0.05	10	58	116	1160	
B	0.3	0.05	10	27	56	560	
C	0.4	0.05	10	16	32	320	
D	0.2	0.07	10	65	132	1320	
E	0.3	0.07	10	30	60	600	
F	0.4	0.07	10	17	36	360	
G	0.2	0.05	5	96	192	960	
H	0.3	0.05	5	43	88	440	
I	0.4	0.05	5	25	52	260	
J	0.2	0.07	5	102	204	1020	
K	0.3	0.07	5	46	92	460	
L	0.4	0.07	5	27	56	280	
M	0.3	0.20	10	50	100	1000	
N	0.4	0.20	10	29	60	600	

## 8. STATISTICAL METHODS

### 8.1 General Considerations

Except for specialized analyses, Stata 12.0 or later will be used for conducting study analyses.

As a cluster randomized trial, SHINE outcomes, in general, will be analyzed using statistical methods that account for within-cluster correlation. With a relatively large number of clusters (212), and for some analyses, only a few events or prevalent cases per cluster, generalized estimating equations (GEE) estimation provides an ideal approach to data analysis. We will use the Stata GEE regression procedure, with exchangeable correlation structure within each cluster, for both continuous and

discrete response variables. Primary analyses will contain two dummy variables representing each intervention in this 2x2 factorial study; they will be unadjusted for other covariates. A log link function with Bernoulli response will be used for prevalence outcomes, to aid in interpretability by directly producing risk ratio estimates. If coefficients do not converge, a Poisson response can be used, as the robust variance estimation will adjust for the Poisson's slightly larger variance specification.

Although this study is not powered to detect a statistical interaction effect between the two interventions, it will be estimated. If there is a statistically important interaction (defined as  $p < 0.05$  from a Wald test), or a sizeable point estimate (defined as a RR of  $> 2$  or  $< 0.5$  when comparing either of the two ratios of ratios: e.g. if the RR of stunting for WASH vs. SOC is 0.9 and the RR for WASH+IYCF vs. IYCF is 0.42, then  $0.9/0.42 > 2.0$  and we would consider this point estimate to be sizeable; or, for a continuous outcome, when a difference of differences is greater than 0.25 SD) we will also present results using a regression model with three terms to represent the four study arms. We recognize, however, that with our continuous primary outcomes, and the relatively large sample size, we may see interactions that are statistically significant but do little to change the substantive interpretation of the effects of the two interventions.

For IYCF and WASH effect modification analyses, statistical significance will be assessed by multiplying the dummy variable for whichever intervention is being considered by the continuous/scale version of the potential modifier; the latter may then be categorized for communication purposes.

The case-control sub-studies (e.g. Risk Factors for miscarriage, stillbirth, preterm delivery, small for gestational age (SGA) at birth, SGA+preterm, and neonatal death) will be analyzed via conditional logistic regression models. Because of the conditioning on the case-control sets, and the fact there will rarely be more than one case per cluster, there will be little opportunity for intra-cluster correlation to be exhibited.

For the Environmental Enteric Dysfunction Sub-Study, questions which address the impact of the WASH intervention will use analyses that adjust for clustering, as per the GEE regression approach described above. This will be the same for the ECD substudy.

## **8.2 Definition of Analysis Sets**

The main analysis sets are not strictly intention-to-treat (ITT) in that we are not collecting outcomes on all those who have been randomized, as randomization occurred at the cluster level. Thus, the main analyses are *modified*; but they are ITT in terms of not having restrictions for having received the full scheduled intervention(s). Note that we do not have a Primary Per Protocol set, as the per protocol subsets are all secondary or tertiary.

### **8.2.1. Primary modified Intention-to-Treat:**

These analyses are all based on children born to women who were HIV-negative in pregnancy. It covers data collected from consent to participate in the study to the child's 18-month visit. Residence at time of consent determines study arm for these analyses. If women are mistakenly enrolled more than once, they will be counted in the trial arm corresponding to their residence at last enrolment. Loss to follow up, in terms of the CONSORT diagram, is anything that keeps a child's 18-mo. length or haemoglobin from being measured—refusal, permanent move outside Zimbabwe are expected to be among the main reasons. This analysis compares 18-month length-for-age and haemoglobin concentration across arms according to the mother's assignment at enrollment, among all those born alive and who had an 18-month measurement.

### **8.2.2. Secondary Modified Per Protocol analyses:**

Secondary modified per protocol analyses will be conducted with these restrictions:

#### **WASH intervention**

Higher Fidelity: received all 5 WASH modules and 12-month review

Lower Fidelity: received less than all 5 WASH modules and 12-month review

#### **IYCF intervention**

Higher Fidelity: received all 5 IYCF modules and 12-month review

Lower Fidelity: received less than all 5 IYCF modules and 12-month review

#### **Note:**

The comparator group for the WASH Higher Fidelity group will be those who received all visits with these product codes: ANC-1, ANC-2, EPI-2, FPM-1, FPM-2, FPM-6.

The comparator group for the IYCF Higher Fidelity group will be those who received all visits with these product codes: FPM-2, FPM-3, EPI-4, FPM-4, EPI-5, FPM-6.

Comparator groups for the Lower Fidelity groups are defined analogously.

One more set of analyses will be made with the additional restriction of excluding all those who enrolled before 1 November 2013, to allow for the early anomalies in latrine construction.

In sensitivity analyses, children born to women who were HIV-negative during pregnancy but HIV-positive at a subsequent postnatal visit (i.e. seroconverters) will be removed. Two further sensitivity analyses will restrict analysis to children whose primary outcomes (length and haemoglobin) were measured during the ideal visit window (76-80 weeks of age), and up to 24 months of age.

### **8.2.3. Tertiary Other Per Protocol:**

Various other subsets may be defined according to stricter adherence or receipt measures.

## **8.3 Trial Outcome Measures**

### **8.3.1 Main trial outcomes**

The outcomes of the main trial are shown below. Details of analysis approach are shown in section 9.

#### *Primary outcomes*

- Length-for-age Z score at 18 months
- Hemoglobin at 18 months

#### *Secondary outcomes*

- Stunting (LAZ <-2) at 18 months
- Severe stunting (LAZ <-3) at 18 months
- Anemia (hemoglobin <105 g/L) at 18 months
- Severe anemia (hemoglobin <70 g/L) at 18 months
- Weight-for-age Z score (WAZ) at 18 months
- Underweight (WAZ <-2) at 18 months
- Weight-for-length Z score (WLZ) at 18 months
- Wasting (WLZ <-2) at 18 months
- Head circumference-for-age Z score at 18 months
- Mid-upper arm circumference-for-age Z score at 18 months



- 7 day maternal recall of child diarrhea, dysentery and acute respiratory infection at 12 and 18 months
- Mortality through 18 months

#### *Tertiary outcomes\**

- Anthropometry (LAZ, WAZ, WLZ, MUAC, head circumference) at 1, 3, 6 and 12 months
- Hemoglobin at 3, 6 and 12 months (EED substudy only)
- Incidence and prevalence of diarrhea
- Persistent diarrhea
- Incidence and prevalence of acute respiratory infection
- Incidence and prevalence of fever
- All-cause and cause-specific hospitalization
- All-cause and cause-specific clinic attendance
- Adverse events and serious adverse events related to trial interventions

\*Not all tertiary outcomes will be presented in the main trial manuscript; further information regarding tertiary analyses is presented in Table 5.

A separate analysis will be undertaken for infants born to HIV-positive mothers.

### **8.3.2 Early child development substudy**

The early child development substudy was added to the trial after enrolment had begun (once extra funding was secured), and the assessment is at 24 months of age. Children turning 102 weeks of age from March 7, 2016 to April 30, 2017 were eligible to join, as explained in section 8.2.4. The ECD substudy has the following outcomes:

#### *Primary outcomes*

- Motor, language, social and cognitive development as assessed by the Malawi Development Assessment Tool (MDAT).
- Specific language development as assessed by the McArthur Bates Communication Developmental Inventory.
- Executive function as assessed by the A not B and delayed inhibition tests.

#### *Secondary outcome*

- Grammar as assessed by the McArthur Bates Communication Developmental Inventory.

#### *Tertiary outcome*

- Disability and behavior as assessed by an adaptation of the Washington tool.

The primary analyses will be based on children born to women who were all HIV negative during pregnancy. It will cover data collected from consent to participate in the study to the child's 24 month visit. Since the ECD visit was added after the trial had begun, this visit was not included in the original informed consent process and consent is sought at or after the 18 month visit, from March 7, 2016 onwards; loss to follow up prior to or following the 18 month visit will therefore prevent a child from having their 24 month child development measured. The analysis compares 24 month MDAT, McArthur Bates CDI, A not B, Delayed Inhibition, disability and behaviour across arms according to mother's treatment arm assignment at enrollment, among all those born alive and eligible between March 7, 2016 and April 30, 2017 who had a 24 month measurement. Since ECD will be measured in a subgroup of children turning 24 months after March 7, 2016, we will compare baseline maternal and infant variables between those on whom 18 month endpoints were measured who do enroll and do not enroll in the ECD substudy.

Children with severe motor, visual, hearing or learning impairments as determined by the Washington screening tool will be excluded from all aspects of the ECD analysis. The MDAT score will be analysed both as a raw score and Z-score but the raw score will provide the primary comparison for publication. The A not B test will only be reported among those who completed the full set of 10 tests. The McArthur Bates CDI, which has been validated in Shona for this study, will only be reported for children in Shona-speaking households.

The same modified ITT and per protocol analyses will be undertaken for the ECD outcome as described in 8.2.1 and 8.2.2, including a sensitivity analysis that excludes infants born to mothers who seroconvert during follow-up.

A separate analysis will be undertaken for children born to HIV-positive mothers (see **Table 5**).

### **8.3.3 Other trial outcomes**

The full list of trial objectives have been previously reported (SHINE Trial Team, Clin Infect Dis 2015; available at [https://academic.oup.com/cid/issue/61/suppl\\_7](https://academic.oup.com/cid/issue/61/suppl_7)) and analysis plans are outlined in Table 5.

### **8.3.4 Environmental Enteric Dysfunction (EED) substudy:**

These analyses will be based on children enrolled into the Environmental Enteric Dysfunction (EED) substudy. The study comprises children born to mothers who were HIV-negative during pregnancy; children were enrolled into the EED substudy at their 32 week visit, their 1 month postpartum visit, or as soon as possible thereafter among infants who reached this age interval from May 1, 2014 through to the end of the trial. In addition, we attempted to enroll all infants of HIV-positive mothers, even those who were older during this period.

A total of 1167 HIV-unexposed and 491 HIV-exposed infants were enrolled. From these infants, samples of stool, saliva, blood, and urine before and after the Lactulose-Mannitol test dose were collected at 1, 3, 6, 12, and 18 months (the LM test was not done at 1 month). These biological specimens are being analyzed in the laboratory for biomarkers of EED and hematopoiesis including: I-FABP, IGF-1, sCD14, CRP, AGP, kynurenine:tryptophan ratio, citrulline, hepcidin, sTFR, ferritin from plasma; hemoglobin from whole blood; and neopterin, myeloperoxidase, alpha-1 antitrypsin and REG-1B from stool.

Analyses are outlined in **Table 5**.

### **8.3.5 Oral vaccine immunogenicity substudy**

Oral vaccines significantly underperform in developing compared to developed countries, and this is plausibly related to the intestinal environment (EED, diarrhea, enteropathogen carriage and skewed microbiota), so it is rational that a WASH intervention might improve oral vaccine immunogenicity. We will capitalize on the introduction of Rotarix at 6 and 10 weeks in the EPI schedule in Zimbabwe, to evaluate the impact of the WASH intervention on rotavirus vaccine immunogenicity at 3 months of age among a subgroup of infants in the EED substudy. A sample size of 516 children with available samples at 1 and 3 months of age (321 non-WASH from 87 clusters and 195 WASH from 66 clusters) provides power of 87% to detect a 15% difference in rotavirus seroconversion between WASH and non-WASH infants, with alpha of 0.05 and k of 0.2. The analysis plan is outlined in **Table 5**.

## 8.4 Measurements and Definitions

A number of parameters will be assessed in this trial that could be measured or defined in a number of ways. **Table 3** lists definitions that will be used in the study. Some of these may be subject to change if more accurate or less costly approaches to measurement become available during the study period. We expect there may be substantial departure from the EED definitions below as the field evolves. We may utilise two already-published definitions, followed by a search for a robust set of variables that discriminate among treatment arms.

**Table 3. Definitions used in SHINE**

Parameter	Definition/Assessment Method
Environmental Enteric Dysfunction in Pregnant Women	Assessed through domains of the hypothesized causal pathway*
Environmental Enteric Dysfunction in Infants	Assessed through domains of the hypothesized causal pathway*
Intestinal absorption and enterocyte mass*	Assessed by mannitol recovery and plasma citrulline
Gut <u>Inflammation</u> in women and infants*	Increased concentrations of fecal neopterin, alpha-1 antitrypsin and myeloperoxidase [including Kosek's score].
Enterocyte damage in women and infants*	Assessed by increased concentrations of intestinal fatty acid binding protein (I-FABP)
Intestinal regeneration in infants*	Assessed by increased fecal concentrations of REG-1B (infants only)
Intestinal barrier function in women and infants*	Assessed by lactulose recovery and fecal alpha-1 antitrypsin
Gut <u>microbial translocation</u> in women and infants*	Increased concentrations of plasma lipopolysaccharide (women only), and soluble CD14
Systemic inflammation in women and infants*	Elevated serum levels of CRP and AGP, kynurenine:tryptophan ratio (and T-cell activation in a subgroup of women and infants)
Infant <u>growth hormone axis</u> *	Depressed plasma levels of insulin like growth factor-1 (IGF-1)
<u>Aflatoxin exposure</u> in women and infants	Detectable AFB1-lysine in plasma, or AFM1 in urine
<u>Fumonisin exposure</u> in women and infants	Detectable FB1 in urine
<u>Deoxynivalenol exposure</u> in women and infants	Detectable DON in urine
<u>Zearalenone exposure</u> in women and infants	Detectable zearalenone in urine
<u>Ochratoxin A exposure</u> in women and infants	Detectable ochratoxin A in urine
<u>T-2 exposure</u> in women and infants	Detectable T-2 in urine
Maternal and infant intestinal microbiota	16S rRNA and whole genome sequencing of DNA and RNA from stool to define the composition and function of the microbial community that inhabits the human intestine.
Maternal anemia	Hemoglobin < 120 g/L as assessed using the Hemocue hemoglobinometer
Maternal severe anemia	Hemoglobin <70 g/L as assessed using the Hemocue hemoglobinometer
Maternal iron status	Plasma ferritin level adjusted for CRP and AGP
Infant anemia	Hemoglobin < 105 g/L as assessed using the Hemocue hemoglobinometer
Infant severe anemia	Hemoglobin < 70 g/L as assessed using the Hemocue hemoglobinometer
Infant rotavirus immunogenicity	Measurement of rotavirus IgA titre in plasma

Miscarriage	Fetal loss prior to 28 gestational weeks
Stillbirth	Fetal loss after 28 gestational weeks
Low birth weight	Infant born weighing <2500 g within 72 hours following delivery
Preterm birth	Infant born prior to completing 37 weeks gestation
Small for gestational age	Infant with weight at birth less than the 10th percentile of Intergrowth fetal/newborn growth standards.
Stunted for a given age Severely stunted for a given age	LAZ < -2 Z-score (WHO 2006) LAZ < -3 Z-score (WHO 2006)
Diarrhea	Passage of 3 or more loose or watery stools in a 24 hour period, as measured by maternal recall.
Dysentery	Passage of blood or mucus in the stools, as measured by maternal recall.
Acute respiratory infection	Fast or difficult breathing, as measured by maternal recall.
Fever	Maternal report of fever or hot body.
IYCF indicators	A set of 7 indicators published by WHO (WHO 2010 )
WASH indicators	Structured observations of key behaviors, and hardware/environment observations to assess WASH behavioral uptake: latrine use (presence of fecal matter in the homestead, a well trodden path to the latrine), handwashing (presence of water in the tippy-tap), water treatment (test for residual chlorine in water) and presence of a protected play area
Nutributter supplementation	Distribution measures and maternal report of feeding the index child and not sharing with other children
Social support for mothering	Assessed using questions adapted from published instruments (Cohen 1985; Sherbourne 1991) and pilot-tested in Shona, comprising material, informational, and emotional support.
Maternal access/control of resources	Questions adapted from Gates-Funded Alive & Thrive Project survey, and pilot-tested in Shona
Maternal depression	Assessed with the Edinburgh Postnatal Depression Scale, which has been validated in Shona with cut-offs defined by Chibanda <i>et al.</i>
Maternal stress	Elevated salivary cortisol concentration
Maternal roles, priorities and time	Novel set of questions developed and pilot-tested in Shona by our team
Maternal perceived physical health	Assessed using an adaption of the SF-36
Mothering self-efficacy	Assessed using questions adapted from published instruments (Dumka 1996; Gilmore 2009) and pilot-tested in Shona

Fidelity of intervention delivery	Extent to which study interventions are delivered as designed/intended. Assessed through review of supervision and VHW records to establish timing of VHW visits and adherence to implementation protocols.
Village Health Worker capacity to deliver randomized interventions	VHWs' motivational and supervisory characteristics assessed using questions adapted from IFPRI-World Vision instruments, published instruments and our formative research. Knowledge tests used to assess knowledge acquisition and the extent of knowledge sharing with mothers.
Early child development (Motor, language, social and cognitive development)	Malawi Development Assessment Tool score (continuous measure in 4 sections – gross motor (GM), Fine motor (FM), language (Lang) and Social plus a total score)
Specific language development	McArthur Bates Communication Developmental Inventory (overall score – continuous measure as primary outcome). Measure of the specific expressive language abilities of the child in Shona (those living in households where Shona is spoken rarely or never will be excluded). A series of questions about grammar skills will be reported as secondary outcomes.
Executive function	A not B (score out of 10) and delayed Inhibition tests (continuous measure of time). Measure of the ability to memorise and maintain object permanence and measure of the ability to wait prior to being provided with a reward.
Child functioning, disability and behavioural questionnaire	Measurement of disability and behavioural problems as provided by the Washington group – categorical – reported within domains (note those with 'a lot of difficulty' or 'cannot do at all' for vision, hearing, walking or learning will be excluded from the ECD assessment).
Home environment (FCI)	Family Care Inventory (continuous measure in 3 sections – Measure of the amount of stimulation the child has in the home.
Maternal Child Interaction	Observation of Maternal Child Interaction
Child cortisol level	Salivary cortisol concentration
Observation of behavior of child and mother	Observational tool from Bayley III (continuous score)

\*Since there is no case definition or single biomarker of EED, it will be assessed by evaluating domains of the hypothesized causal pathway of EED, outlined in more detail in Prendergast AJ et al, Clin Infect Dis 2015; 61(S7):S726-32, and may employ additional biomarkers in each domain depending on emerging data from other studies.

### 8.5 Analyses Adjusted for Baseline Covariates

For the primary and secondary outcomes, besides the main unadjusted analyses, we will conduct analyses adjusted for baseline covariates (and clustering, in a GEE model as described) in order to account for residual confounding and/or improve power. The variables to be considered for inclusion are (these have data on >90%, or use a category that includes Missing):

#### Maternal

Age  
Height

MUAC  
Years of completed schooling  
Marital status  
Parity  
Employment status  
Religion (3 dummies for Apostolic, Other Christian, and Other religion, no religion or missing)  
Maternal capabilities  
Maternal hemoglobin  
CD4 count and PMTCT exposure during pregnancy (HIV-infected women only)

#### Household

HH Coping Strategies Index - HH receives food aid from government or other organization  
Any latrine  
Improved latrine  
Open defecation  
Feces observed in yard  
Improved floor  
Time to drinking water  
Owns chickens  
Livestock observed in house  
Household size  
Wealth quintile (5 dummies for the 5 quintiles and Missing)

#### Infant

Low birthweight (2 dummies for <2500g, >=2500g, Missing)  
Gender  
Preterm

#### Study

Data collector (only for outcomes relying on measured infant length or weight or early child development assessment)  
Calendar quarter of study (dummies)

For each outcome, each of the above covariates will be assessed in a bivariate analysis, and those covariates that meet a minimum requirement will be entered into multivariable analyses. A minimum requirement for dichotomous outcomes is:  $p < 0.2$  or  $RR > 2.0$  or  $< 0.5$ . For continuous outcomes:  $p < 0.2$  or difference  $> 0.25$  SD. For these analyses, dichotomous (cut at the median) versions of continuous covariates will be used, but then if selected, the continuous version will be used in the next stage. For covariates represented by multiple dummy variables, only the overall Wald test will be used. The exception is the Data collector (DC) variable: it will be assessed via a likelihood ratio test in a random effects model with random intercepts for cluster and for DC. If  $p < 0.2$ , then a GEE model with DC represented by dummies will be run, and the DCs with the 10 furthest-outlying estimates will be entered into the next stage (to then be represented by 9 dummies).

The next stage will consist of a backward stepwise selection procedure, with  $p < 0.2$  to remove, and  $p < 0.2$  to remain.

Further exceptions may be made when there is insufficient data for stable estimation of coefficients, which may occur in subgroup analyses or for relatively rare outcomes. For continuous outcomes, we will ensure there are at least 5 outcome measures for every parameter to be estimated. For dichotomous outcomes, we will ensure there are at least 10 events (prevalent conditions) for every parameter to be estimated. If these conditions are not met, the first covariates to be dropped are DC, then calendar quarter, then the next least statistically significant covariates.

## 8.6 Subgroup analyses

For the primary and secondary outcomes, a subgroup analysis will be performed by infant sex. Statistical interaction terms will be evaluated to look for differential intervention impact in these subgroups.

## 8.7 Sensitivity analyses

For the primary and secondary outcomes, the following sensitivity analyses will be conducted:

- i) Removing infants of mothers who enrolled in the trial prior to Oct 1, 2013, because receipt of some latrines was delayed by implementation challenges.
- ii) Removing infants born to women who seroconverted in pregnancy (i.e. pregnancy status was HIV-negative and 18 month status was HIV-positive).
- iii) Restricting the analysis to infants whose endline measurements occurred during the ideal window (76-80 weeks of age).
- iv) Removing infants whose endline measurements occurred after 24 months of age.

For the ECD substudy, only sensitivity analysis ii) will be conducted.

## 9. STUDY ANALYSIS PLAN

Analyses will be performed according to the statistical principles of **Section 8**. Missing data will be counted as missing, and given its own category (if categorical) or noted in the appropriate table (if continuous). Data for each of the four study arms will be given, and pairs combined to analyze the two interventions.

**Table 4. Baseline and Follow up Summaries (i.e., some will be in the CONSORT diagram)**

Summary: by each analysis set, where relevant, and by study arm
Number of participants enrolled
Status of enrolled participants—both mothers and children—at Study Visits: Birth, End of pregnancy, 3, 6, 12, 18, 24 months post-partum (status: evaluated; dropped out; lost to follow up; dead)
Demographics and Baseline Characteristics:
Maternal age (mean, SD)
Maternal height (mean, SD)
Maternal MUAC (mean, SD)
Maternal haemoglobin (mean, SD)
Years of schooling (median, IQR)
Maternal parity (mean, SD)
Marital status, % married
Employment status, % employed
Percentage mothers meeting minimum dietary diversity
Maternal capabilities
Religion – distribution into categories: apostolic, other Christian, other religion)

Percentage of households meeting minimum dietary diversity score  
 Wealth index – distributed into quintiles  
 Coping strategies index, median (IQR)  
 Any latrine, percentage  
 Improved latrine, percentage  
 Open defecation among household members, percentage  
 Feces observed in yard, percentage  
 Improved floor, percentage  
 Time to drinking water, median, IQR  
 Treat water, percentage  
 Own chickens, percentage  
 Number of chickens, median, IQR  
 Livestock in house, percentage  
 Household size, median, IQR  
  
 Infant sex, percentage female  
 Infant birthweight, mean (SD)  
 Low birth weight, percentage <2500g  
 Gestational age, mean (SD)  
 Preterm, percentage <37 weeks  
 Delivery place, percentage institutional delivery  
 Delivery mode, percentage vaginal delivery

**Table 5. Primary and Secondary Specific Aims (from Protocol version 18, May 20, 2016) with their corresponding Analysis Plan**

Primary Specific Aims	Primary Analysis Plans
<p>1. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among <b>HIV negative</b> women and continued through 18 months post partum, on <b>length (primary outcome) and stunting (secondary outcome, defined as LAZ &lt;-2.0)</b> of rural Zimbabwean infants at 18 months of age?</p>	<p><b><i>The primary analyses compare 18-month length-for-age Z-score and haemoglobin concentration across each of the two intervention pairs of study arms among infants born to mothers who were HIV-negative during pregnancy</i></b></p> <p>By ‘18’ month we mean up to 30 months of age (end of allowable visit window) for LAZ, and for haemoglobin. Additional sensitivity analyses will include data only collected during the ideal window of the 18 month visit (76-80 weeks) and only up to 24 months for LAZ, as outlined in section 8.7. A conversion factor will be used for any lengths that are measured after 24 months of age so as to give an appropriate Z-score for recumbent lengths.</p> <p>These two analyses, and their corresponding analyses for stunting and anemia, will also be run adjusting for baseline variables that may be related to both enrollment into the study and the outcomes. These will be baseline characteristics of the mother, infant, and other household-level variables that, in univariate analyses, are related to study outcomes with a risk ratio of at least 2 (or &lt;0.5 depending on coding), or a p-value (adjusted for within-cluster correlation) of &lt;0.05. Further details are in Section 8.5.</p> <p>Intermediate measures of our primary outcomes for babies of HIV-negative women: LAZ at 1, 3, 6 and 12mo and</p>
<p>2. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among <b>HIV negative</b> women and continued through 18 months post partum, on <b>haemoglobin (primary outcome) and anemia (secondary outcome, defined as haemoglobin &lt;105 g/L)</b> of rural Zimbabwean infants at 18 months of age?</p>	



	<p>Haemoglobin concentration at 3, 6, 12 mo (EED substudy infants only) will be examined prior to publication of the primary results; we will not plan on including them, unless there is something rather striking that should be presented. We may, however, show a graph of growth over time by arm.</p> <p>Other secondary outcomes are listed in section 8.3.1.</p> <p>Deaths among those live-born up to 18 mo, by maternal baseline HIV status, will be analyzed by Kaplan-Meier at 24 months (using Greenwood variance estimator) if fewer than 10% of the deaths occur in multiple-death clusters. Else, we will fit a Cox regression with gamma frailty.</p> <p>Subgroup and sensitivity analyses are defined in sections 8.6 and 8.7</p> <p>We will present uptake variables measured at 12 months of age and intervention delivery data for infants included in the final 18 mo endpoint analysis.</p>
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Secondary Specific Aims	Secondary Analysis Plans
<p>1. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among <b>HIV positive</b> women and continued through 18 months post partum, on <b>length (primary outcome) and stunting (secondary outcome, defined as LAZ &lt;-2.0)</b> of rural Zimbabwean infants at 18 months of age?</p>	<p><b><i>The first secondary outcomes compare 18-month length-for-age Z-score and haemoglobin concentration across each of the two intervention pairs of study arms among infants born to mothers who were <u>HIV-positive</u> during pregnancy</i></b></p> <p>These two analyses, and their corresponding analyses for stunting and anemia, will also be run adjusting for baseline variables that may be related to both enrollment into the study and the outcomes. These will be baseline characteristics of the mother and other household-level variables that, in univariate analyses, are related to study outcomes with a risk ratio of at least 2 (or &lt;0.5 depending on coding), or a p-value (adjusted for within-cluster correlation) of &lt;0.05. Further details are in Section 8.5.</p> <p>Other secondary outcomes are listed in section 8.3.1.</p> <p>Deaths among those live-born up to 18 mo, by maternal baseline HIV status, will be analyzed by Kaplan-Meier at 24 months (using Greenwood variance estimator) if fewer than 10% of the deaths occur in multiple-death clusters. Else, we will fit a Cox regression with gamma frailty.</p> <p>Subgroup and sensitivity analyses are defined in sections 8.6 and 8.7</p> <p>We will present uptake variables measured at 12 months of age and intervention delivery data for infants included in the final 18 mo endpoint analysis.</p> <p>All analyses will stratify infants as HIV-exposed uninfected versus HIV-infected or HIV-unknown.</p>
<p>2. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF), when these interventions are initiated in early pregnancy among <b>HIV positive</b> women and continued through 18 months post partum, on <b>haemoglobin (primary outcome) and anemia (secondary outcome, defined as haemoglobin &lt;105 g/L)</b> of rural Zimbabwean infants at 18 months of age?</p>	

<p>3. What is the effect of the IYCF intervention on uptake of improved infant feeding practices by maternal/infant HIV status, specifically:</p> <p>a. Infant diet quality as assessed by WHO IYCF indicators (WHO 2010)</p> <p>b. Infant nutrient intake from complementary foods assessed by 24 hour dietary recall</p> <p>c. Appropriate use of Nutributter from 6 to 18 months</p>	<p>a). Infant diet quality indicators (WHO IYCF indicators) assessed from entire cohort at 3, 6, 12, and 18 months. Analyses combining all time-points to get 'average' over study period. Analyze separately by maternal/infant HIV status</p> <p>b). Infant nutrient intake from complementary food assessed by 24 hour dietary recall at one time point (12 mo) among a subgroup of 200 children (50/treatment group). Run separately by maternal/infant HIV status.</p>
<p>4. What is the effect of the WASH intervention on the 5 key behaviors it promotes by maternal/infant HIV status?</p> <p>a. Proper disposal of animal and human feces</p> <p>b. Handwashing with soap (HWWS) after fecal contact</p> <p>c. Point-of-use chlorination of drinking water</p> <p>d. Protecting children from ingestion of dirt and feces</p> <p>e. Feeding baby freshly prepared foods, or reheating leftover food</p>	<p>At 3, 6, 12, and 18 months. Analyses combining all time-points to get 'average' over study period. Separately by maternal HIV status during pregnancy.</p>
<p>5. For each randomized intervention (IYCF and WASH), is the impact on <b>stunting</b> of children at 18 months of age modified by the following factors in addition to maternal/infant HIV status?:</p> <p>a. Household and community socioeconomic and demographic characteristics. For example, maternal age, size of the household, socioeconomic status, etc.</p> <p>b. Village Health Worker (VHW) performance assessed by degree of transfer of correct knowledge to participating mothers and the quality of VHW interpersonal approach</p> <p>c. Maternal capacities (1) social support for mothering or the mother's social networks, (2) access to and control of resources, (3) depression and stress, (4) roles, priorities and time, (5) perceived physical health, and (6) mothering self-efficacy</p>	<p>Child stunted at 18 months: IYCF and WASH impacts, separately, with product term to assess interaction between intervention and</p> <p>a. Maternal age; children ever born; SES</p> <p>b. Baseline maternal capabilities index</p> <p>c. Maternal HIV status during pregnancy</p>
<p>6. For each randomized intervention (IYCF and WASH), is the impact on <b>anemia</b> at 18 months of age modified by the following factors in addition to maternal/infant HIV status?</p> <p>a. Household and community socioeconomic and demographic characteristics</p> <p>b. Village Health Worker (VHW) performance assessed by degree of transfer of correct knowledge to participating mothers and the quality of VHW interpersonal approach</p> <p>c. Maternal capacities (1) social support for mothering or the mother's social networks, (2) access to and control of resources, (3) depression and stress (4) roles, priorities and time, (5) perceived physical health, and (6) mothering self-efficacy</p>	<p>Child anemic (Hb&lt;105 g/L) at 18 months: IYCF and WASH impacts, separately, with product term to assess interaction between intervention and</p> <p>a. Maternal age; children ever born; SES</p> <p>b. Baseline maternal capabilities index</p> <p>c. Maternal HIV status during pregnancy</p>
<p>7. What is the effect of the WASH intervention on diarrhea prevalence among infants 0-18 months of age? Is this different among children born to HIV positive and negative mothers?</p>	<p>1-week diarrhea, dysentery and respiratory disease prevalence at 1, 3, 6, 12, 18 months (whole cohort). GEE analyses combining all time-points to get 'average' over study period. Separately, and with product term to assess interaction between intervention and maternal HIV status during pregnancy.</p> <p>Also, among the EED substudy group from whom morbidity diaries are available: cumulative prevalence of diarrhea.</p> <p>Algorithms developed by the Nepal team (Tielsch et al) will be</p>

	used for episode definition from the EED diaries.
8. What is the prevalence of exclusive breastfeeding among all infants enrolled in the trial?	Prevalence of exclusive breastfeeding among all infants enrolled in the trial.
9. What is the prevalence of anemia among pregnant women at 10-26 weeks by HIV, mycotoxin and schistosomiasis status?	Prevalence of anemia among pregnant women at baseline by HIV, schistosomiasis status, and “mycotoxin status”
10. What is the prevalence of schistosomiasis among pregnant women living in areas of high prevalence among school-aged children?	Prevalence (at baseline and at 32 weeks) and incidence (between baseline and 32 weeks of schistosomiasis (defined as dipstick hematuria and detectable ova, with microscopy taking precedence) among pregnant women
11. What is the prevalence of mycotoxin exposure among pregnant women?	Prevalence of mycotoxin exposure (defined in Table 3) among pregnant women (at baseline, 32 weeks, or both)
12. What is the prevalence and severity of EED among pregnant women at 10-26 weeks and 30-34 weeks gestation by maternal HIV and mycotoxin status, as assessed by: <ul style="list-style-type: none"> <li>a. Maternal <u>gut inflammation</u></li> <li>b. Maternal <u>gut permeability</u></li> <li>c. Maternal <u>gut microbial translocation</u></li> <li>d. Maternal <u>systemic immune activation</u></li> <li>e. Maternal <u>growth hormone axis</u></li> </ul>	Prevalence and severity of EED among pregnant women at baseline by maternal baseline HIV and “mycotoxin status”. Prevalence will be assessed if emerging data from other studies defines a dichotomous outcome for EED, although this is unlikely. Severity will be assessed by looking at distributions of the continuous versions of these measures. Domains assessed by the minimum list of indicators in Table 3, which define the pathological processes underlying EED. NOTE: There are emerging biomarkers of EED which will be evaluated, since there is no consensus case definition or current gold standard biomarker.
13. What is the association between maternal EED and infant birth outcomes by maternal/infant HIV status?	Separate models for the outcomes, which are described in section 10. Primary covariate(s): Maternal EED status. Stratified by maternal HIV status during pregnancy. Adjusted for all potential confounders (these may differ by outcome) including SES measures, the total number limited as described in Section 10.
14. What is the association between maternal schistosomiasis and infant birth outcomes by maternal/infant HIV status, and are these associations mediated through inflammation?	Separate models for the outcomes, which are described in section 9. Stratified by maternal HIV status during pregnancy. Primary covariate: maternal schistosomiasis at baseline; and at either baseline or 32 weeks. Adjusted for all potential confounders including SES measures.
15. What is the association between maternal mycotoxin exposure and infant birth outcomes by maternal/infant HIV status?	Separate models for the outcomes, which are described in section 9. Stratified by maternal HIV status during pregnancy. Primary covariate: maternal “mycotoxin exposure” as defined in Table 3. Adjusted for all potential confounders including SES measures.
16. What is the association between infant EED and early child development at 24 months of age, stratified by maternal HIV status?	Infant EED will be measured at 1, 3, 6, 12 and 18 months as described in section 8.3.4. Separate models for the ECD outcomes, which are described in section 8.3.2. Adjusted for all potential confounders including SES measures.
17. What are the independent and combined effects of a	The primary and secondary ECD substudy outcomes are listed

<p>package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among <b>HIV negative women</b> and continued through 18 months post-partum, on <b>early child development</b> of rural Zimbabwean infants at 24 months of age?</p>	<p>in section 8.3.2. Analyses of continuous variables (MDAT, A not B, delayed inhibition and MacArthur Bates score; all primary ECD outcomes) to be carried out as for LAZ and hemoglobin. These analyses will also be run adjusting for baseline variables that may be related to both enrollment into the study and the outcomes. These will be baseline characteristics of the mother, infant, and other household-level variables that, in univariate analyses, are related to study outcomes with a risk ratio of at least 2 (or &lt;0.5 depending on coding), or a p-value (adjusted for within-cluster correlation) of &lt;0.05. Further details are in Section 8.5. Analysis of dichotomous variables (grammar skills, secondary outcome) to be carried out as for stunting and anemia.</p> <p>Behaviour and disability are tertiary outcomes, using an adaptation of the Washington screening tool. Because this has not previously been used as an outcome measure, these analyses will be more exploratory and will evaluate the impact of the randomized interventions on measures of disability and behavior which may be categorized into domains, and may utilize summative scores or item response approaches.</p> <p>Subgroup and sensitivity analyses are defined in sections 8.6 and 8.7</p> <p>We will present uptake variables measured at 12 months of age and intervention delivery data for infants included in the final 24 mo endpoint analysis.</p>
<p>18. What are the independent and combined effects of a package of interventions to improve household water, sanitation, and hygiene (WASH) and a package of interventions to improve infant feeding practices (IYCF – Infant and Young Child Feeding), when these interventions are initiated during early pregnancy among <b>HIV positive women</b> and continued through 18 months post-partum, on <b>early child development</b> of rural Zimbabwean infants at 24 months of age?</p>	<p>The analysis will be carried out as above, but among infants born to HIV-positive women who were evaluated for ECD at 24 months of age. The same primary, secondary and tertiary ECD outcomes will be used as for infants born to HIV-negative women.</p> <p>All analyses will stratify infants as HIV-exposed uninfected versus HIV-infected or HIV-unknown.</p> <p>Subgroup and sensitivity analyses are defined in sections 8.6 and 8.7</p> <p>We will present uptake variables measured at 12 months of age and intervention delivery data for infants included in the final 24 mo endpoint analysis.</p>
<p>19. Additional analyses of the independent and combined effects of the WASH and IYCF interventions.</p>	<p>These analyses do not have specific articulated research questions, but some will have relatively high priority. All are listed in section 8.3.1 as tertiary trial outcomes.</p> <p>To determine the independent and combined effects of improved household WASH and improved IYCF on:</p> <ul style="list-style-type: none"> <li>• Diarrhea incidence and prevalence (EED substudy, by daily morbidity diary)</li> </ul>

		<ul style="list-style-type: none"> <li>• Persistent diarrhea (&gt;2wks) (EED substudy, by daily morbidity diary)</li> <li>• ARI incidence and prevalence (EED substudy, by daily morbidity diary)</li> <li>• Febrile illness incidence and prevalence (EED substudy, by daily morbidity diary)</li> <li>• WAZ, WHZ, MUAC, Head circumference at 1, 3, 6, 12 mo, and LAZ at 1, 3, 6 and 12mo</li> <li>• Haemoglobin concentration at 3, 6, 12 mo (EED substudy infants only)</li> <li>• All-cause and cause-specific hospitalizations (self-report) via Poisson regression models.</li> <li>• All-cause and cause-specific clinic attendance via Poisson regression models.</li> <li>• Proportion alive and non-stunted at 18 mo. (complement of proportion dead OR stunted at 18 mo.), by maternal baseline HIV status</li> <li>• All Adverse events related to the trial interventions.</li> <li>• All Serious Adverse Events related to the trial interventions.</li> </ul>
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Environmental Enteric Dysfunction Substudy	Analysis Plan
<p>1. What are the effects of the WASH intervention on each of the following domains of the hypothesized biomedical causal pathway between poor sanitation/hygiene and child <u>stunting</u> at 1, 3, 6, 12, and 18 months of age?</p> <ol style="list-style-type: none"> <li>Infant <u>gut inflammation</u></li> <li>Infant <u>gut permeability</u></li> <li>Infant <u>gut microbial translocation</u></li> <li>Infant <u>systemic immune activation</u></li> <li>Infant <u>growth hormone axis</u></li> </ol>	<p>Analyses to be carried out as for LAZ, using appropriately transformed versions of the biomarkers of interest as the dependent variables to determine the impact of the randomized intervention on each biomarker, or biomarker scores as outlined in Table 3.</p>
<p>2. For the domains listed in #1, what is the association between the variability in each upstream domain with that of the downstream domains (upstream=a and downstream=e along a continuum from a to e) among all children and stratified by intervention group?</p>	<p>To test the hypothesis that EED mediates the effect of WASH on stunting and anemia, an intent-to-treat analysis will be carried out on each domain in the causal pathway. We will use GEE equations to construct prediction equations for child LAZ at 18 months. We will multiply regression coefficient estimates for the treatment effect of the WASH intervention on EED exposure among children in the WASH arm of the trial by those partial regression coefficients for the association of EED with child LAZ among children in the SOC arm to attain the estimated effect of the WASH intervention on child LAZ at 18 mo mediated by EED. Variability in each domain of the pathway will be characterized either as a continuous function or as categories. For some domains we will explore the computation of summary indices. Thereafter, for each link in the pathway, we will study the association between variability in the upstream domains/variables and variability in the subsequent domains /variables. Second, we will calculate maximum likelihood estimates of these same parameters using a path analysis approach. Using path analyses we will compare the relative strength of EED and diarrhea in mediating the effect of improved WASH on stunting and anemia.</p>

<p>3. What is the relative importance of EED vs. diarrhea in mediating the effects of the WASH intervention on stunting?</p>	<p>Path analysis to assess the relative contributions of diarrhea and EED to stunting. We will use two complementary approaches to assess the mediating effects of child diarrhea and environmental enteric dysfunction (EED) on attained linear growth (i.e., length-for-age Z-score (LAZ)) at 18 months. We will first use generalized estimating equations to construct prediction equations for child LAZ at 18 months. We will multiply regression coefficient estimates for the treatment effect of the WASH intervention on EED exposure among children in the “WASH” arm of the trial by those partial regression coefficients for the association of EED with child LAZ among children in the “Standard of Care” arm to attain the estimated effect of the WASH intervention on child LAZ at 18 months as mediated by EED. We will carry out these same analyses to assess mediation by diarrhea. Second, we will calculate maximum likelihood estimates of these same parameters using a path analysis approach. We will estimate the indirect effect of the WASH intervention on child LAZ as mediated by EED by multiplying the path coefficients from the WASH intervention to EED, and from EED to child LAZ in our hypothesized causal model. The same approach will be taken to assess mediation by diarrhea. In both approaches, the products of the coefficients of each set of analyses will be compared to assess the magnitude and statistical significance of each pathway.</p>
<p>4. What are the effects of the WASH intervention on each of the following domains of the hypothesized biomedical causal pathway between poor sanitation/hygiene and child <u>anemia</u> at 1, 3, 6, 12, and 18 months of age?</p> <ol style="list-style-type: none"> <li>Infant <u>gut inflammation</u></li> <li>Infant <u>gut permeability</u></li> <li>Infant gut <u>microbial translocation</u></li> <li>Infant systemic <u>immune activation</u></li> <li>Infant hepcidin production as assessed by plasma hepcidin levels</li> <li>Infant soluble transferrin receptor (sTRF) concentration</li> <li>Infant ferritin concentration</li> <li>Infant CRP</li> <li>Infant AGP</li> </ol>	<p>Analyses to be carried out as for LAZ, using appropriately transformed versions of the biomarkers of interest as the dependent variables.</p>
<p>5. Are the associations observed in specific aims 1-4 above modified by HIV status of the mother/infant?</p>	<p>Carried out by adding interaction terms.</p>
<p>6. What is the effect of the WASH intervention on diarrhea incidence and duration among infants 0-18 months of age, stratified by maternal/infant HIV status?</p>	<p>Incidence analyses will be carried out with Poisson regressions. Note that analyzing duration only among those with episodes breaks the randomization. Rather, incidence of long-duration episodes will be modeled via Poisson regression analyses, with a cutoff of 2 weeks.</p>
<p>7. What are the associations between inflammatory markers (CRP, AGP), iron status and anemia among pregnant women at 10-26 weeks and 30-34 weeks gestation according to their HIV status?</p>	<p>This can be handled as a set of Spearman rank correlations between the two time points, using continuous measures of iron status and haemoglobin.</p>
<p>8. What are the associations between maternal and infant EED, and between maternal EED and infant postnatal</p>	<p>First are correlation analyses; second is a binary response log-link model of stunting as a function of maternal EED or its</p>

stunting?	indicators.
9. Is EED more severe among infants who are mixed fed compared to infants who are exclusively or predominantly breast fed, stratified by maternal/infant HIV status?	EED or its appropriately transformed biomarkers will be the response variable as a function of breastfeeding status. We will also use data from TAC card analysis on enteropathogen carriage and from microbiota analyses to describe differences in the intestinal milieu between infants according to breastfeeding status.
10. What is the prevalence of mycotoxin exposure among infants between birth and 18 months, and what is the association between mycotoxin exposure, EED and stunting between birth and 18 months?	<p>The first step will be to describe the prevalence and severity of mycotoxin exposures in Zimbabwean infants by age. For aflatoxin we will also describe the prevalence and severity of exposure by season. This descriptive work will also allow us to find the best form for the mycotoxin exposure variables in subsequent analysis, which may include:</p> <ul style="list-style-type: none"> <li>• Prevalence of values above a cutoff (dichotomous)</li> <li>• Mycotoxin concentrations as continuous variables, which may require some transformation to meet normality criteria. Alternatively, quartiles or quintiles of exposure.</li> <li>• Summary exposures over time, for example, a summary of aflatoxin exposure across the 4 time points of infancy that we will assess.</li> <li>• For the 200 infants with DON and FUM measurements, a summary variable of multiple mycotoxin exposures; e.g. whether 0, 1, 2, or 3 mycotoxins were present.</li> </ul> <p>Our primary hypothesis is that mycotoxin exposure will be associated with linear growth restriction in infants. We will test this hypothesis using multivariate regression models, with linear growth as the outcome variable and mycotoxin exposure as the independent variable. Linear growth will be modeled as both a continuous variable (attained HAZ score) and, using logistic regression, as a dichotomous variable (stunted vs. not stunted). The WHO growth reference will be used to calculate Z scores. Models will take the form:</p> <p>(1) Child linear growth= <math>B_0 + \beta_1[\text{mycotoxin}] + \beta_x[\text{covariates}] + e</math>,</p> <p>Where covariates will include: district, socio-demographic variables of the mother and household, household diet diversity and food security, and breastfeeding status (exclusive/partial/not breastfed) Randomized group allocation will also be included because Nutributter will be provided as a supplement to 2 of the study arms.</p> <p>Initially, we will build models for each individual time point of mycotoxin assessment. Finally, we will use repeated measures multivariate analysis of variance (MANOVA) as follows:</p> <p>(2) Child linear growth= <math>B_0 + \beta_1[\text{mycotoxin}] + \beta_2[\text{timepoint}] + \beta_3[\text{mycotoxin} * \text{timepoint}] + \beta_x[\text{covariates}] + e</math></p> <p>Our second hypothesis is that mycotoxin exposure will be</p>



	<p>associated with gut dysfunction (i.e. EED). The EED biomarkers used in this analysis will be consistent with the overall SHINE trial. Our statistical approach will be the same as described above for the outcome of stunting.</p> <p>To test whether EED, diarrhea and decreased appetite mediate the association between mycotoxins and stunting, we will use the approach of MacKinnon, Fairchild &amp; Fritz. The following series of models will be tested:</p> <p>(3) Child linear growth= <math>B_0 + \beta_1[\text{mycotoxin}] + \beta_x[\text{covariates}] + e_1</math></p> <p>(4) Child linear growth= <math>B_{01} + \beta_1'[\text{mycotoxin}] + \beta_2[\text{mediator}] + \beta_x[\text{covariates}] + e_2</math></p> <p>(5) Mediator= <math>B_{02} + \beta_1[\text{mycotoxin}] + \beta_x[\text{covariates}] + e_3</math>,</p> <p>Where the mediators are EED, diarrhea and decreased food intake. Each mediator will be assessed through a separate set of equations at each individual time point of assessment.</p> <p>To take advantage of our longitudinal data, we will also use latent-growth modeling to examine whether the “growth” in mycotoxin exposure over time affects the growth trajectory of the mediator, which affects the growth trajectory of child linear growth. The general mediation equations (3-5) will be used to explore the potential individual mediations of EED, diarrhea and reduced appetite.</p> <p>We will use structural equation modeling (SEM) to compare the relative strength of association between mycotoxins and stunting through the hypothesized pathways of decreased food intake, EED, and diarrhea. After exploring individual mediators, we will test whether the above variables are interrelated through a set of linear relationships by examining the variances and covariances of the variables.</p> <p>The final phase of our analysis will explore whether the mycotoxin-stunting (and mycotoxin-EED) relationships are independent of WASH variables. We hypothesize that the effect of mycotoxins will be independent of WASH, meaning that the relation of mycotoxins to stunting will be observed similarly across all randomized treatment arms within SHINE. To explore this question, we will run the models from the above analysis for the WASH and non-WASH groups separately, and examine whether the beta coefficients of interest change substantially (e.g. by &gt;15%). If they appear to change substantially we will test this statistically by introducing a WASH*mycotoxin interaction term in the models.</p>
11. What is the impact of the maternal microbiota, infant breastfeeding practices and the WASH intervention on early (0-6 months) intestinal microbiota development and on infant growth between 0 to 6 months?	Regression models with either markers of infant intestinal microbiota status at 6 months or of LAZ at 6 months as the response variable will be fit with measures of maternal microbiota, infant BF practices, and WASH intervention as covariates, with the 3 2-way interactions among these covariates.
12. What is the impact of the early microbiota, the IYCF	Regression models with markers of early infant intestinal



intervention and the WASH intervention on late intestinal microbiota development and on infant growth between 6-18 months?	microbiota status at 18 months as the response variables will be fit with measures of early microbiota, IYCF and WASH interventions as the covariates, with the 3 2-way interactions among these covariates. Infant LAZ at 18 months will be regressed on infant LAZ at 6 months and measures of early infant intestinal microbiota status and IYCF and WASH interventions, with the 3 2-way interactions among these covariates.
<p>13. What is the association between infant EED and infant rotavirus vaccine and polio vaccine immunogenicity?</p> <p>14. What are the effects of the WASH intervention on infant rotavirus vaccine and polio vaccine immunogenicity?</p>	In 321 infants randomized to non-WASH arms, we will evaluate measures of vaccine immunogenicity, using each intestinal factor (enteropathogen score, incident diarrhoea and biomarkers of EED) at 12 weeks as explanatory variables in a multiple linear regression model. Both the effect on GMC and proportion above protective levels (with log-transformed response variable—linear regression; and binary cutoff variable—log-binomial models) will be analysed. Among 516 infants (195 randomized to WASH, 321 randomized to non-WASH) we will compare enteropathogen score, incident diarrhoea and biomarkers of EED to evaluate the impact of the randomized intervention, then compare rotavirus immunogenicity (GMT and seroconversion) between arms, using a generalised estimating equation regression model with binary outcome and logit link function and exchangeable correlation structure, to account for cluster-level correlation. Combining infants randomised to WASH and non-WASH arms, we will categorise fully vaccinated infants as good/poor responders then explore which baseline variables identify infants at risk of poor rotavirus vaccine responses, using a generalised estimating equation regression model with binary outcome and logit link function and exchangeable correlation structure.
15. What are the associations between aflatoxin exposure and maternal EED among pregnant women at 10-26 weeks gestation according to their HIV status?	Within each HIV status group, measures of maternal EED at baseline (10-26 weeks gestation) will be regressed on a summary measure of total aflatoxin exposure at 10-26 weeks.

<b>Risk Factors for SGA/Preterm Birth Substudy</b>	<b>Analysis Plan</b>
1. What is the association between maternal EED and SGA/premature delivery stratified by maternal/infant HIV status?	See section 10 for full description of case-control studies
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and SGA/premature delivery stratified by maternal/infant HIV status?	See section 10 for full description of case-control studies
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and SGA/premature delivery stratified by maternal/infant HIV status?	See section 10 for full description of case-control studies
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and SGA/premature delivery stratified by maternal/infant HIV status?	See section 10 for full description of case-control studies

<b>Risk Factors for Stillbirth Substudy</b>	<b>Analysis Plan</b>
1. What is the association between maternal EED and	See section 10 for full description of case-control studies

stillbirth stratified by HIV status?	
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?	See section 10 for full description of case-control studies
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?	See section 10 for full description of case-control studies
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and stillbirth stratified by maternal HIV status?	See section 10 for full description of case-control studies

<b>Risk Factors for Miscarriage Substudy</b>	<b>Analysis Plan</b>
1. What is the incidence of miscarriage, and what are the epidemiological exposures associated with miscarriage in rural Zimbabwean women?	See section 10 for full description of case-control studies
2. What is the association between maternal EED and miscarriage stratified by HIV status?	See section 10 for full description of case-control studies
3. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?	See section 10 for full description of case-control studies
4. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?	See section 10 for full description of case-control studies
5. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and miscarriage stratified by maternal HIV status?	See section 10 for full description of case-control studies

<b>Risk Factors for Neonatal Death Substudy</b>	<b>Analysis Plan</b>
1. What is the association between maternal EED and neonatal death stratified by HIV status?	See section 10 for full description of case-control studies
2. What is the association between maternal anemia measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?	See section 10 for full description of case-control studies
3. What is the association between maternal schistosomiasis measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?	See section 10 for full description of case-control studies
4. What is the association between maternal mycotoxin exposure measured at 10-26 weeks and 30-34 weeks gestation and neonatal death stratified by maternal HIV status?	See section 10 for full description of case-control studies

## 10. CASE-CONTROL STUDIES WITHIN SHINE

**10.1 Overview:** A series of case-control studies of adverse birth outcomes will be conducted within SHINE, designed as 1:2 matched case-control studies for miscarriage, stillbirth and neonatal death, and as a four-group study of SGA and preterm compared to AGA/term infants.

Cases and controls for each study will be constructed independently of the other studies, such that a mother-baby pair may be selected into more than one set of cases and/or more than one set of

controls. However, the overarching approach to selecting cases and controls will be consistent across this series of studies.

## 10.2 Case events of interest:

- Miscarriage (fetal loss <28 weeks)
- Antepartum stillbirth (fetal loss >28 weeks)
- Preterm (defined as <37 weeks)
- SGA (defined as weight <10<sup>th</sup> centile for gestational age using Intergrowth reference standards)
- SGA and preterm
- Neonatal death (defined as deaths in first 28 days of life
  - stratified by early (<7 days) and late (8-28 days)

## 10.3 Inclusion criteria

To be eligible for inclusion in any of the case-control studies, mother-infant pairs need to have the following minimum dataset:

- Known pregnancy outcome
- Known gestational age
- Known maternal HIV status during pregnancy
- Baseline visit done

Within this pool of women, we will select cases and controls for each exposure and birth outcome combination based on availability of the exposure of interest data:

- Maternal schistosomiasis microscopy result from baseline (for schistosomiasis analysis)
- >200uL archived maternal plasma from baseline (for mycotoxin analysis)
- >200uL archived maternal plasma, LM urine and/or stool from baseline (for maternal EED analysis)

Mother-infant pairs will be excluded if maternal HIV status is unknown, if a baseline visit was not done, if the pregnancy outcome is unknown, if the infant gestational age is implausible or if data on the exposure of interest are not available.

## 10.4 Miscarriage substudy

**Case definition:** All women with fetal loss before 28 gestational weeks, stratified by maternal HIV status in pregnancy. We may further stratify miscarriages by <24 weeks and 24-28 weeks depending on number of events.

**Control Definition:** Two pregnancies will be selected per case among mothers who did not experience fetal loss by the time of the event, stratified by maternal HIV status in pregnancy.

**Case selection:** To be included in the substudy as a case, women must have documented fetal loss before 28 weeks and inclusion criteria as outlined in section 1.3.

**Control Selection:** To be included in the substudy as a control, women must have a known pregnancy outcome and be documented not to have fetal loss at or before the time of the case

event, together with the inclusion criteria outlined in section 10.3. Controls will be selected as cases are identified, using incidence density sampling, using the following approach:

- For each case, two windows will be assigned – one for the date of the event and one for the date of the baseline visit. Each will be allocated to one-week gestational age windows (8-9 weeks, 9-10 weeks, 10-11 weeks etc).
- A pool of potential controls will be identified, who have the same HIV status in pregnancy as the case, who have not had fetal loss at or before the time of the case event (i.e. in that one-week GA window) and who had a baseline visit within the same one-week GA window as the case.
- Two controls will then be randomly selected from all women in that control pool, matched by intervention arm.
- This selection procedure will match controls with cases based upon gestational age at incidence, and gestational age at baseline visit. If there are no controls within the one-week bands, we will widen the baseline visit window by 1 week and continue doing that until we find 2 suitable controls.

### 10.5 Stillbirth substudy

**Case definition:** All women with fetal loss after 28 gestational weeks, stratified by maternal HIV status in pregnancy.

**Control Definition:** Two pregnancies will be selected per case among mothers who did not experience fetal loss by the time of the event, stratified by maternal HIV status in pregnancy and matched on intervention arm.

**Case selection:** To be included in the substudy as a case, women must have documented fetal loss after 28 gestational weeks and inclusion criteria as outlined in section 1.3.

**Control Selection:** To be included in the substudy as a control, women must have documented not to have fetal loss before or at the time of the case event, and fulfill inclusion criteria as outlined in section 10.3. Controls will be selected as cases are identified using incidence density sampling, using the following approach:

- For each case, the date of the event and the date of the baseline visit will be allocated to one-week gestational age windows (8-9 weeks, 9-10 weeks, 10-11 weeks etc).
- A pool of potential controls will be identified, who have the same HIV status in pregnancy as the case, who have not had fetal loss at or before the time of the case event (i.e. in that one-week GA window) and who had a baseline visit within the same one-week GA window as the case.
- Two controls will then be randomly selected from all women in that control pool, matched on intervention arm.
- This selection procedure will match controls with cases based upon gestational age at incidence, and gestational age at baseline visit. If there are no controls within the one-week bands, we will widen the baseline visit window by 1 week and continue doing that until we find 2 suitable controls.

Exposures will be measured at baseline for all participants in the case-control studies for pregnancy loss. For a subset of stillbirths we can also conduct a study to examine the effect of “exposure” at 32 weeks gestation on the outcome.

We may look at the continuum of fetal loss by combining the miscarriage and stillbirth case-control studies, or stratifying by <24 weeks, 24-28 weeks and >28 weeks.

We may conduct a sensitivity analysis where we remove stillbirths that were likely to be antepartum, based on SAE and/or maternal reports of the infant appearance at birth (fresh vs macerated), but we will not restrict case selection using these criteria because they can be unreliable predictors of time since fetal death (KJ Gold et al., Int J Gynaecol Obstet 2014).

## 10.6 Neonatal death substudy

**Case definition:** All women whose liveborn infant died in the first 28 days of life, stratified by maternal HIV status in pregnancy, and stratified by early (days 1-7) and late (days 8-28) neonatal death.

**Control Definition:** Two pregnancies will be selected per case among mothers who have a liveborn infant who did not die by the time of the case event, stratified by maternal HIV status in pregnancy and matched by infant sex and intervention arm.

**Case selection:** To be included in the substudy as a case, women must have a documented liveborn infant with neonatal death, known age at time of event, and fulfill inclusion criteria as outlined in section 1.3.

**Control Selection:** To be included in the substudy as a control, women must have documented to have a liveborn infant who did not die at or before the time of the case event, have known infant age at time of the case event, and fulfill inclusion criteria as outlined in section 10.3. Controls will be selected as cases are identified using incidence density sampling, using the following approach:

- For each case, the date of the death will be allocated to one of two windows (0-7 days, 8-28 days), and the date of the baseline visit will be allocated to one-week GA windows (8-9 weeks, 9-10 weeks, 10-11 weeks etc).
- A pool of potential controls will be identified, who have the same HIV status in pregnancy as the case, who have not had neonatal death before or in the window when the case event occurred and who had a baseline visit within the same one-week GA window as the case.
- Two controls will then be randomly selected from all women in that control pool, matched by infant sex and intervention arm.
- This selection procedure will match controls with cases based upon infant age at incidence, and gestational age at baseline visit. If there are no controls within the one-week bands, we will widen the baseline visit window by 1 week and continue doing that until we find 2 suitable controls.

We may also want to look at the 32 weeks gestation visit data and results if available, but will match based on baseline visit.

## 10.7 SGA/preterm substudy

**Case definition:** All women whose liveborn infant was preterm/AGA, preterm/SGA or term/SGA, stratified by maternal HIV status in pregnancy.

**Control Definition:** Women whose liveborn infant was term/AGA, stratified by maternal HIV status in pregnancy and matched by infant sex and intervention arm.

**Case selection:** To be included in the substudy as a case, women must have documented liveborn infant with SGA or preterm or both, and fulfill inclusion criteria as outlined in section 1.3. All 3 case groups (SGA/term, AGA/preterm and SGA/preterm) will be pooled for this substudy, and controls selected as below.

**Control Selection:** To be included in the substudy as a control, women must have documented to have a liveborn infant who was AGA and term, have known infant gestational age at time of the case event (to enable calculation of GA and SGA), and fulfill inclusion criteria as outlined in section 10.3. Controls will be selected as cases are identified using incidence density sampling and group matching, using the following approach:

- For each case, the date of the event will be allocated to one-week GA windows (28-29 weeks, 29-30 weeks, 30-31 weeks etc) and the date of baseline visit allocated to one-week GA windows (8-9 weeks, 9-10 weeks, 10-11 weeks etc).
- A pool of potential controls will be identified, who have the same HIV status in pregnancy as the case, who have infants born AGA and term in the window when the case event occurred and who had a baseline visit within the same one-week GA window as the case.
- A group of controls will then be randomly selected from all women in that control pool, using  $\sqrt{k}$  x number of cases (where k is the number of case groups, in this case 3) as the estimate of control group size, matched by infant sex and intervention arm.
- This selection procedure will match one control group with cases from 3 groups based upon infant age at incidence, and gestational age at baseline visit. If there are no controls within the one-week bands, we will widen the baseline visit window by 1 week and continue doing that until we find sufficient suitable controls.

We may also want to look at the 32 weeks gestation visit data and results if available, but will match based on baseline visit.

## 10.8 Analysis approach

The primary analysis will be among HIV-negative cases and controls; a subsequent analysis will pool HIV-infected and HIV-uninfected women and include HIV status as a covariate. Conditional logistic regression models will be fit, adjusted for potential confounders including SES measures. The primary covariates of interest will be maternal EED, schistosomiasis, anemia and mycotoxin exposure. Alternatively (and more likely), for maternal EED, we will perform a robust search for sets of discriminating variables, e.g. taking the top 6 variables that are the intersection of those found by random forests and SCAD approaches. In general, the number of covariates in any given model will be limited by the number of cases, under the rule of 10 cases required per estimated covariate.

## 11. CLASSIFICATION AND REPORTING OF ADVERSE OUTCOMES

### 11.1 Adverse Event and Serious Adverse Event reporting

An Adverse Event (AE) is defined as any untoward medical occurrence in an enrolled study participant, regardless of its relationship to trial activities.

Serious Adverse Events (SAEs) are defined by the ICH Harmonised Tripartite Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting (1994), i.e. an incident that involved an enrolled study participant and includes one or more of the following:

1. Results in death of a study participant

2. Is life threatening to a study participant (i.e. at risk of death at the time of the event)
3. Requires unplanned hospitalization, OR prolongation of existing hospitalization of a study participant
4. Results in persistent or significant disability/incapacity of a study participant
5. Is a congenital anomaly or birth defect of a study baby
6. Is any other important medical event considered serious by the investigator of a study participant (e.g. anaphylaxis requiring emergency treatment but not resulting in hospitalization). An important medical event should carry a real, not hypothetical, risk of one of the above events (1-5) if prompt medical action were not taken.

AEs and SAEs will be reported on an Event Form. The staff member reporting the event will complete part 1 of the Event Form and submit it to the Research Nurse Supervisor or Nurse Intervention Manager at the Hub the same day. The RNS/NIM will review all Event Forms within 24 hours of the form being completed, and will fill in Part 2 of the form. The RNS/NIM will pass the form to the Field Data Supervisor in the hub, who will create an electronic record of the event on the database, within 24 hours of receiving the form; this will generate an Event number.

The Compliance Team at Zvitambo will review all Event forms within 48 hours of receipt. The compliance team will adjudicate the event and check Part 1 and Part 2 of the form, then provide a summary of the event(s) and decisions made. Adverse Events and SAEs will be judged as either unlikely, uncertainly, probably or definitely related to the study interventions or procedures.

Since this is a community-based trial, we anticipate a large number of AEs and SAEs that are unrelated to trial activities. Because of the rural location and limited means of communication within the study districts, it is unlikely that the trial team will find out about most AEs and SAEs in real time.

#### **11.1.1 Reporting of events to the local IRB (MRCZ)**

Events will be reported to MRCZ according to the following guidelines:

##### **1. AEs and SAEs identified outside of scheduled study visits:**

Where the study team first learns about an AE or SAE outside of a scheduled study visit (e.g. report to the field hub by a VHW, or other staff member), a home visit will be made as soon as possible to collect information about the event. An event form will be completed by the field staff, reviewed by the compliance team at Zvitambo head office, and reported to MRCZ under the expedited reporting time-frame (3 days for SAE, 7 days for AE).

##### **2. AEs and SAEs identified during scheduled study visits:**

Where the study team first learns about an AE or SAE through a questionnaire administered during a scheduled study visit, information will be collected about the event as part of routine data capture. All events that resulted in a clinic visit will subsequently be reported in a table (split by type of AE) to MRCZ every quarter (3 monthly). For the subgroup of AEs that qualify as SAEs, a narrative report will be completed for each one, together with an MRCZ Event Reporting form, and linked to the Table by an SAE number, so that MRCZ staff will easily be able to review all SAE narratives and link them to the reporting table. The rationale for reporting all AEs that led to clinic attendance is to capture those SAEs that are likely to be grade 3 or 4 (serious or life threatening). Sufficient data will not be captured to enable grading of every AE symptom according to DAIDS tables.

During routine home visits, we may learn of AEs and SAEs that are not part of our routine data collection (eg social harm). In these instances, events will be reported with a full narrative according to the expedited MRCZ timeframe for AEs and SAEs.

The potential adverse events that may be related to our interventions (although have not been described to date by the manufacturers or distributors) are reactions to Nutributter and accidental ingestion of Waterguard. To be certain that we detect any reactions to Nutributter, we will ask a series of questions during home visits, from 6 months of age, to all mothers whose babies are randomized to receive Nutributter. Any adverse events will be followed up with a full AE/SAE narrative in the field, and expedited reporting to MRCZ and the DSMB. Any cases of accidental Water Guard ingestion will similarly undergo expedited reporting to MRCZ and the DSMB.

**3. Reporting of abnormal clinical investigations conducted as part of study protocol:**

Abnormal results which are grade 3 or 4 adverse events according to DAIDS tables (or qualify for referral according to Zimbabwean guidelines, where not covered by DAIDS tables), will be reported to MRCZ on the quarterly table. These will be graded as shown in Tables 6 and 7.

**Table 6: Classification of adverse events for women enrolled in SHINE**

<b>Parameter</b>	<b>Grade 3 Severe*</b>	<b>Grade 4 Potentially life threatening*</b>
Haemoglobin (known HIV positive woman)	6.5 – 7.4 g/dL	<6.5 g/dL
Haemoglobin (known HIV negative woman, or HIV status unknown)	7.0 – 8.9 g/dL	<7.0 g/dL
Hypertension	≥180 mmHg systolic OR ≥110 mmHg diastolic	Life-threatening consequences (e.g.g malignant hypertension) or hospitalization indicated (other than clinic visit)
Hypotension	Symptomatic, requiring admission for IV fluids	Shock requiring vasopressors or mechanical assistance to maintain blood pressure
Malnutrition	MUAC <230 mm	MUAC <190 mm OR bilateral pitting oedema
Proteinuria	4+ on urinary dipstick testing	NA



Hematuria	Gross hematuria	Hematuria requiring transfusion
Maternal depression	Alteration in personality-behaviour causing inability to perform usual social and functional activities	Behaviour potentially harmful to self or others (e.g. suicidal and homicidal ideation or attempt, acute psychosis) OR causing inability to perform basic self-care functions.
New HIV infection	NA	NA
New urinary schistosomiasis	NA	NA

\*Grading based on Division of AIDS (DAIDS) table for the grading of adverse events (published December 2004, with clarification August 2009), where available, hence safety criteria listed here may differ from trial definitions listed in Table 2. Malnutrition parameters based on Zimbabwe MOHCC criteria for referral for nutritional rehabilitation, taken from 'Malnutrition Management of Acute Malnutrition in Zimbabwe' (Version 1; June 2011).

**Table 7: Classification of adverse events for infants enrolled In SHINE**

<b>Parameter</b>	<b>Grade 3 Severe*</b>	<b>Grade 4 Potentially life threatening*</b>
Haemoglobin (infant aged 22-35 days)	7.0 – 7.9 g/dL	<7.0 g/dL
Haemoglobin (infant aged 36-56 days)	6.0 – 6.9 g/dL	<6.0 g/dL
Haemoglobin (infant or child aged ≥57 days)	7.0 – 8.9 g/dL	<7.0 g/dL
Malnutrition in a child <6 months	Weight-for-length Z-score <-3 OR MUAC <110 mm	Grade 3 criteria PLUS any IMCI danger sign

	Bilateral pedal oedema OR  Infant too weak to feed or suckle effectively	
Malnutrition in a child $\geq 6$ months	Weight-for-height Z-score $< -2$ OR  Bilateral pedal oedema OR  MUAC $< 125$ mm	Grade 3 criteria PLUS any IMCI danger sign
New HIV infection	NA	NA

\*Grading based on Division of AIDS (DAIDS) table for the grading of adverse events (published December 2004, with clarification August 2009), where available, hence safety criteria listed here may differ from trial definitions listed in Table 2. Malnutrition parameters adapted from Zimbabwe MOHCC criteria for referral for nutritional rehabilitation (Malnutrition Management of Acute Malnutrition in Zimbabwe, Version 1; June 2011) and from published data on predictive value of MUAC in infants  $< 6$  mo old (Mwangome 2012).

If any abnormal investigation also fulfils SAE criteria (e.g. severe anemia leading to hospitalization), a narrative report will be completed, together with an MRCZ Event Reporting form, and linked to the Table by an SAE number, so that MRCZ staff will easily be able to review all SAE narratives and link them to the reporting table.

If any mother or child enrolled in the trial is found to be acutely unwell at a scheduled home visit, they will be assessed by the study research nurse as per their training. If a mother or child needs to be referred to clinic for assessment then an AE form will be completed.

#### **11.1.2. Reporting of events to the international IRB (Johns Hopkins University)**

AEs and SAEs that are judged uncertainly, probably or definitely related to study interventions or procedures will be reported to JHU IRB according to their expedited time-frame (within 10 days of the investigators learning of the event). AEs and SAEs that are judged unlikely related to study interventions and procedures will be reported annually to JHU IRB in the Progress Report.

We will also report the following to the JHU IRB within 10 working days of learning of the event: i) information that indicates a change to the risk:benefit ratio of the research; ii) withdrawal from marketing of a product being used (Nutrigrain or Waterguard); iii) changes to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a research participant; iv) incarceration of a participant; v) any event that requires prompt reporting to the sponsor; vi) complaint of a participant where the complaint indicates unexpected risks or the complaint cannot be resolved by the research team; vii) protocol violations (meaning an accidental or unintentional

change to the IRB approved protocol) that placed one or more participants at increased risk, or has the potential to occur again.

#### ***11.1.3 Reporting of events to the DSMB***

Any grade 3 or 4 AEs or SAEs that are related to Nutributter or Water Guard will be reported to the DSMB within 3 days of the investigators learning of the event. All other SAEs (excluding abnormal laboratory results) will be reported to the DSMB every quarter, split by trial arm.

#### ***11.1.4 Definition of Safety Outcomes of Special Concern***

Any of the following:

- SAE
- Peanut allergy—anaphylaxis
- Water Guard consumption
- All-cause hospitalization